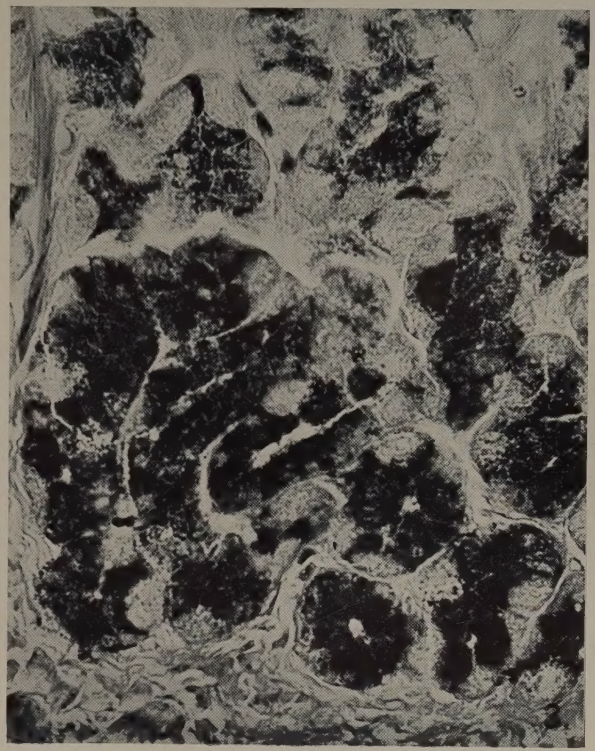

4

THE ORIENTATION OF VENOUS VALVES IN RELATION TO BODY SURFACES

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TWO TEXT FIGURES AND FOUR PLATES (TWELVE FIGURES)

INTRODUCTION

In the course of study of human venous valves, I have observed a seemingly constant orientation of the valves and their cusps to the body surfaces. Experiment has indicated that this orientation serves a useful purpose. Few observations have previously been made on the tension effects of the perivenous tissue in controlling or determining the shape of the vein in cross section, and even fewer on the orientation of the valves. Fabricius (1603) believed that successive pairs of valves are arranged at right angles to each other; and to prove this he showed in his plates veins turned inside out, comparing the position of the valves to the position of the branches of a twig of Verbena. For clarity Fabricius' drawings and explanatory figure legends are here reproduced (fig. 1). In 1854 de l'Aulnoit published his researches on venous valves. He also turned a vein inside out and agreed with Fabricius that the valves are arranged alternately at right angles to one another, but stated that this arrangement was not perfectly symmetrical. Both Fabricius and de l'Aulnoit removed long stretches of vein from the body, so that their observations are open to question, because of the possibility, by such a procedure, of twisting the vein on its axis.

Concerning the relation of the vein to the surrounding tissue, de l'Aulnoit remarks, moreover, that the connective

tissue sheath enclosing the vein gives support to the vessel wall, thereby counteracting immoderate dilatation of the vein and preventing valvular insufficiency. The factor of support given to veins by the periadventitial connective tissue is enlarged upon by Bardeleben (1877) and correlated to the histology of veins in various locations. The adherence of the

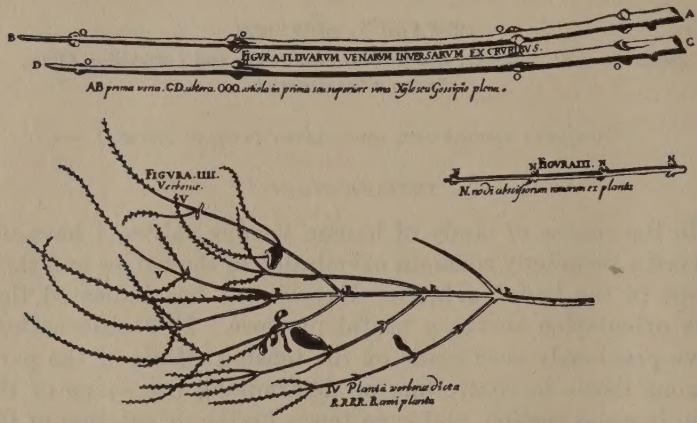

Fig. 1 Venous valves and their orientation as portrayed by Fabricius (1603, 1687). The following legend is adapted from Franklin's ('33) translation of Fabricius' original text.

Figura II presents two veins of the legs, AB and CD, turned inside out to show how the valves O.O.O. are placed in the lumen of the veins. From an inspection of these everted veins one particular thing is clear, namely, that the first and upper valves are situated opposite [at right angle] to those next in succession, like the branches in plants, as will be shown in Figura III.

Figura III, in which nodes of a plant are seen N.N.N. where branches have been cut off. It is placed here, as stated under Figura II, to facilitate comparison between valves and the origin of branches.

Figura IV shows the plant called Verbena. This is the well-known herb from which, in the third figure, the branches have been cut off.

venous walls to aponeuroses, as a means by which the veins are kept open during bending movements of the various parts of the body, is pointed out by Bérard (1830) and by de l'Aulnoit. For the rest, none of the above questions is further amplified in the detailed descriptions of valves by Henle (1876), Sappey (1888), or in the recent work of Kampmeier and Birch ('27).

OBSERVATIONS

The present observations demonstrate that a vein is elliptical in cross section at the site of each valve. Moreover, the major axis of this ellipse is roughly parallel to the plane of the overlying skin. Furthermore, the two valve cusps arise from the long curves of the ellipse, so that the interval between them is also parallel to the overlying compressing skin (figs. 3, 4, 6, 7, 8, 9). This arrangement, which should be designated as the true anatomic position, imparts to the valve a maximum of functional efficiency.

In the limbs the cylinders of skin and fasciae, made tense by their contents, exert a constant elastic force on the deeper structures, and gape when cut. In other words, the skin and fasciae tend constantly to diminish their circumference, and they press inward along their radii. This force is exerted on the collapsible veins and flattens the veins against the underlying muscles and bone. The venous valves are so oriented that the cusps are parallel to the compressing structures or their tangents (figs. 2 B, 3, 4, 6, 7, 8 and 9).

This principle can be demonstrated in both the superficial and deep veins of the upper and lower extremities by preparing cross sections of the cadaver (figs. 3 to 9), or by x-ray visualization in the living (figs. 10 to 14). Occasionally in a cross section of a limb the vein and valve are placed within a triangular niche of fat. In such a case, the fat acting as a semifluid medium, the overlying force flattens the vein against the underlying muscle, and the valve cusps are found parallel to the subjacent muscle, while not exactly parallel to the plane of the skin and fasciae.

The advantage of this relationship of valve to skin becomes evident if one compares the physiologic action of a valve in the anatomic position (fig. 2 B) with that of a valve of purely hypothetical position, placed with its main axis perpendicular to the skin (fig. 2 A). In the latter case, with the valve cusps at right angles to the overlying tangent of skin and fasciae, and parallel to the elastic force of these structures, there would be a tendency for the cusps to become distorted and for their

free edges to be pulled apart when the wall of the vein was compressed and flattened. The valve would obviously become non-efficient and incompetent.

In figure 2 B the actual condition of a typical valve, as demonstrated in the present study, is indicated. The cusps

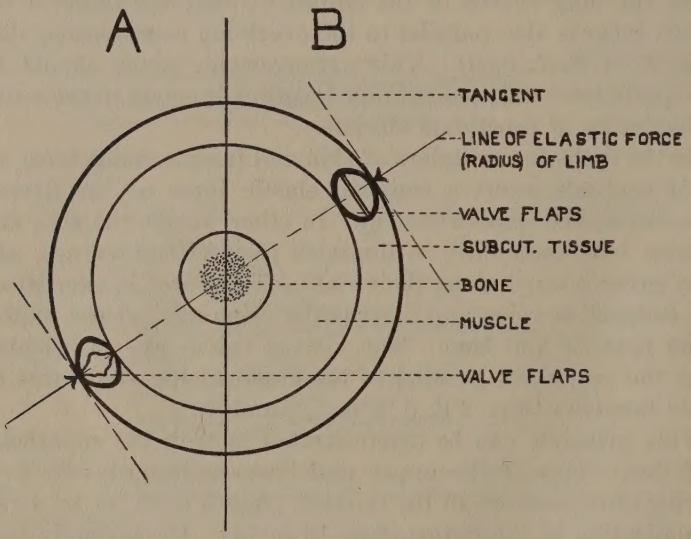

Fig. 2 Diagram to illustrate the effect of compressing forces on a venous valve in an extremity.

In A, the cusps are oriented in non-anatomic position, so that they are perpendicular to the tangent of skin and fasciae, and parallel to the compressing force of these structures. The cusps are distorted by the compression.

In B, the cusps are oriented in the anatomic position, parallel to the tangent of skin and fasciae, and perpendicular to their compressing force. The cusps are accurately approximated by the compression.

are parallel to the tangent of the skin and fasciae and at right angles to the elastic force, and consequently the cusps are pressed against each other. Further, the tendency for lateral widening of the vein puts the free edges of the cusps on the stretch and helps to keep them linear. Thus accurate approximation of the cusps and competency of the valve result.

In this paper reference has been made to bicuspid valves. The question might be raised as to the orientation of unicuspid valves, an example of which is shown in figure 5, V. Unicuspid valves are, however, not nearly so numerous as has been pictured by Fabricius, but they do, of course, occur. In my experience the same principle holds for them as for the bicuspid valves. The single cusp in this type of valve is unusually large, because it effects the closure of the vein in the place of the ordinary two. Moreover, the free margin of the unicuspid valve lies horizontal to the skin. Thus in regard to competency and orientation the unicuspid valve is essentially similar to the bicuspid valve.

The principle described above was tested further by the following simple experiment. Two fresh human femoral veins were excised and laid in grooves in a block of sponge rubber. Then the veins were compressed by a piece of plate glass, one vein being turned so that its valve cusps were parallel to the compressing plate of glass (anatomic position), the second turned so that its cusps were at right angles to the plate of glass (theoretically incorrect position). On attempting to force a stream of water against the valves, the valve which was compressed in the anatomic position was competent and checked the flow of water; while the valve which was compressed in the anatomically incorrect position was incompetent and allowed a retrograde expulsion of water.

The relationship between valve and line of elastic force appears to be adequately and clearly demonstrated by the present study for the veins of the extremities. To what extent a similar relationship between compressing force and orientation of valves exists within the interior of the body is more difficult to observe. In the chest and abdomen the viscera exert pressure against the parietes; while within the visceral mass the pressure is more or less equally distributed in all directions. In the adult there are very few valves in the veins in the thorax and abdomen, and the present investigator has been unable to make conclusive observations regarding the orientation of these valves. Nevertheless, in the case of a

fairly constant valve, the iliac, the cusps are found parallel to the overlying peritoneum and to the underlying muscle (fig. 5). This orientation of the iliac valve, which it has been possible to observe in a number of cadavers, indicates that the relationship of valve to compressing force, demonstrable in the extremities, probably applies equally well to other body surfaces besides the skin.

Examination of the valves in living subjects was made by x-ray visualization involving the injection into the vein of an organic iodide, Skiodan,[1] a technique previously described by the author ('33). The exposure is made during the injection, for if it is delayed, the opacity will be diminished by the dilution and removal of the iodide by the blood stream. To display the valves to best advantage they can be partially closed by impeding the venous flow by a fairly loose tourniquet applied to the upper extremity, or, in the case of the lower extremity, by exposing the limb in the standing position. If the x-ray is taken perpendicular to the skin, the shadows of the cusps of subjacent veins are superimposed, and only the dilatation of the valve sinus will be apparent. However, if the rays are directed tangential to the skin, the cusps will be photographed in profile, forming an inverted 'V.'

Examination of these x-rays confirms the findings obtained from anatomical dissection. In figure 10, for example, the veins in the front of the cubital region are viewed anteroposteriorly, i.e., along the axis of the line of force, and hence the outlines of the cusps are not apparent. In only one place (fig. 10, V.), where a vein is viewed tangentially beneath the skin, are the V-shaped cusps visible. On the other hand, when a plate is taken of this same region so that the veins underlying the skin are viewed for the most part laterally, i.e., tangential to the furface, the V-shaped apertures of the valves at once become apparent (fig. 11, V.V.).

In figure 12 the great saphenous vein and a collateral of that vein are seen at the knee. This lateral view of the knee shows a valve, V, in each vein, with the cusps parallel to the skin of the back of the knee.

[1] Supplied by Winthrop and Co.

Figure 13 shows the two lesser saphenous veins on the lower leg. No valves are visible in those portions of the veins which lie against the calcaneous where the vein walls are viewed perpendicularly through the skin. Higher up in the photograph, where the veins gain the back of the leg, their walls are seen from the side and hence their cusps are apparent.

Figure 14 is of the popliteal vein showing the V-shaped indentation of the valve cusps brought out by the picture having been taken from the side of the leg.

These observations from x-ray plates substantiate the thesis that the valve cusps are oriented horizontal to the skin in the normal, living subject. Thus study of both the cadaver and the living subject indicates that the valves of the veins of the extremities are definitely oriented in reference to the compressing forces of the surrounding tissues. The results do not confirm the view of either Fabricius or de l'Aulnoit that successive valves are situated opposite or at right angles to one another like the branches of plants.

In conclusion a subsidiary point in regard to the shape of veins and valves requires amplification. The writer has referred to veins at the valve site as being elliptical, a description to which exception might be taken. From the examination of valves both in the cadaver and in the living subject at operation, and by x-ray demonstrations, the present investigator has had ample opportunity to observe that veins of the extremities in situ are elliptical throughout their course and even more so at the site of valves. The only condition under which a vein becomes cylindrical, and consequently round in cross section, is when it has been dissected either in the cadaver or at operation so that it has been released from the surrounding compressing forces.

SUMMARY AND CONCLUSIONS

A seemingly constant orientation of the valves of human veins of the extremities is described. The vein at the site of a valve is elliptical in cross section, the major axis of the ellipse being parallel to the skin or its tangent. Within the vein at the valve site the two cusps arise from the long curves of the ellipse, so that the aperture between their free margins is also parallel to the overlying skin. The advantage of this arrangement is that the compression transmitted to the veins by overlying structures produces secure apposition of the cusps to each other and thereby ensures the competency of the valve. The demonstration of the relationship of venous valves to the overlying skin adduced in this paper is based both upon anatomical dissections and upon x-ray studies of the living subject.

Presumably a similar relationship exists between venous valves and adjacent surface planes in the interior of the body. Actual observation, however, has been extended only to one fairly constant valve in the external iliac vein, in which the cusps are definitely oriented in a plane parallel to the parietal peritoneum.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

Figures 3 to 9. The orientation of venous valves as seen in cross sections of human limbs. The specimens here pictured were taken from fresh cadavers. No attempt was made to fix the tissues. A slip of blackened film was placed beneath the interval of some of the valves to outline them more sharply. V., denotes the valves. It is seen that the cusps of the valves invariably lie horizontal to the overlying skin.

3 Valve in the median cubital vein.

4 Valve in the basilic vein in the middle of the arm (deep portion).

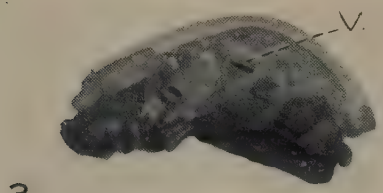
5 Valve in the external iliac vein. In this particular specimen the valve was of the unusual unicuspid type. P is the peritoneum.

6 Valve in the common femoral vein below the entrance of the deep femoral.

7 Valve in the great saphenous vein just before its termination.

8 Valve in the popliteal vein.

9 Valves in the posterior tibial veins. Note the parietal valve, P. V., evidently extending upward from the entrance of a tributary. Such valves occur at the entrance of a small obliquely entering tributary into a much larger vein, and are constituted by an upward prolongation of the side of the vein below the tributary's mouth.



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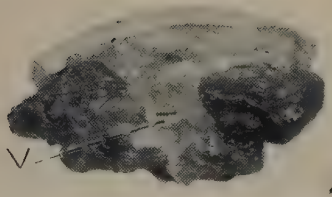

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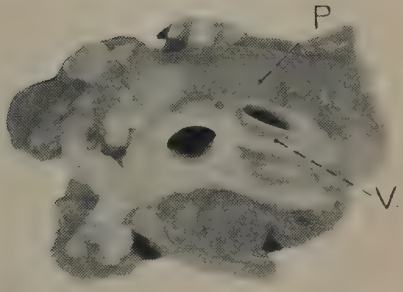

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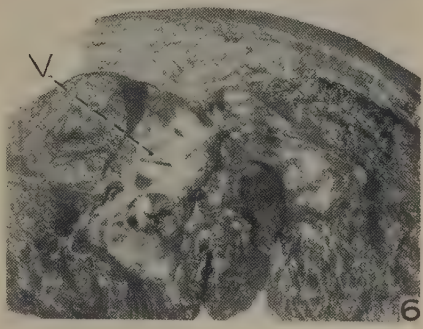

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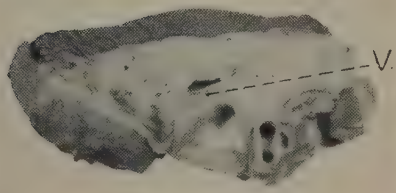

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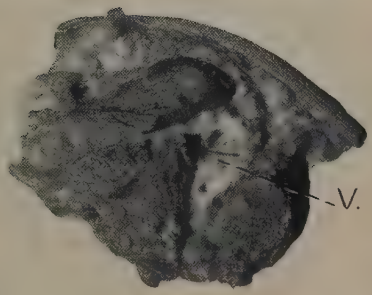

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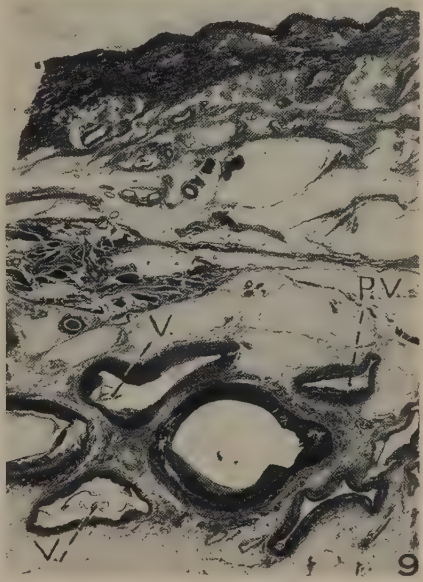

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PLATE 2

EXPLANATION OF FIGURES

Figures 10 and 11. The orientation of valves as seen in roentgenograms of the upper extremity of the living subject.

10 The veins of the cubital region shown in anteroposterior view. Few valves are seen because the line of the view is at right angles to the cusps and their shadows are superimposed. One valve, V., is seen in a vein which runs along the radial border of the forearm and is therefore seen in lateral view.

11 The veins of the same region as figure 10 seen in lateral view. Many valves come into view. They are seen in profile and their cusps are evidently parallel to the overlying skin.

10

11

PLATE 3

EXPLANATION OF FIGURES

Figures 12 and 13. The orientation of valves as seen in roentgenograms of the saphenous veins of the living subject.

12 The great saphenous at the knee and a collateral of that vein. This lateral view of the knee shows a valve, V., in each vein, with its cusps parallel to the skin of the back of the knee.

13 Two lesser saphenous veins on the lower leg. No valves are visible in those portions of the veins which lie against the calcaneus where the valves are viewed in the horizontal position beneath the skin. Higher up in the photograph, as the veins gain the back of the leg, the cusps become apparent (V.) because they are seen in profile.

V.
V.
12

V.
V.
13

PLATE 4

EXPLANATION OF FIGURE

14 The orientation of the valve in the lower part of the popliteal vein shown by roentgenogram in the living subject. L. S. is the lesser saphenous vein, V., is the valve in the popliteal vein, with its two cusps parallel to the skin of the popliteal region and visible because the vein is photographed tangential to the surface.

L.S.
V.
14

ADAPTATION OF THE TRANSPARENT CHAMBER TECHNIQUE TO THE EAR OF THE DOG[1]

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FOUR TEXT FIGURES AND ONE PLATE (FOUR FIGURES)

INTRODUCTION

The transparent chamber method for the study of cells and tissues in the living animal, developed by Dr. Eliot R. Clark and his associates in this laboratory, was devised in order to make possible the same long continued microscopic observations in the mammal which had previously been carried out in the naturally transparent regions of lower vertebrates such as the tadpole's tail and frog's web. (Clark, '09, '12.) The chambers were first perfected for insertion in the ears of rabbits and a number of different varieties of chambers adapted for various types of studies and experimental procedures have been worked out and described (Sandison, '24; Clark, Kirby-Smith, Rex and Williams, '30; Abell and Clark, '32; Williams, '34 a). In addition, a method of inserting a chamber into a skin flap made on the body wall of a rabbit has been developed in order to compare the activity of the blood vessels of the ear with those of another part of the body (Williams, '34 b).

The desirability of extending this method to other mammals is obvious, and this paper presents the results of a successful effort to install transparent chambers in the ears of dogs, including a brief account of the method, some of the similarities and differences in the reactions in the dog as compared with the rabbit, and preliminary observations on the growth and behavior of blood vessels in the dog.

[1] The work in this laboratory on the development of methods for the study of living cells and tissues in transparent chambers in the living mammal, is being aided by a grant from the Rockefeller Foundation.

METHOD

The animals used so far have been beagles, or rabbit hounds. Dogs of this breed are small—the average weight being from 18 to 25 pounds—and may be handled and trained for laboratory purposes with ease. Their large ears, typical of hounds, are eminently suitable for the insertion of chambers and for subsequent attachment to the stage of the microscope for observation. Quiet by nature, these animals may be adapted to laboratory methods without the use of an anesthetic. This characteristic docility appears to be more pronounced in females than in males. The first animal to be used in this series of studies, a dog which was under observation for more than a year, was trained to go directly to the laboratory, when released from the animal house, and to return after the daily observation period. When placed upon the animal board she would lie perfectly quiet, without being tied, remaining in position for more than an hour before becoming restless.

A larger animal board but of similar construction to that used for observations on rabbits and with the same type of head holder was used for these studies. (Clark, Sandison and Hou, '31.)

The chambers used for this first series of studies in the dog were of the 'round table' type, similar to those employed in experiments on rabbits (Clark, et al., '30) with the exception of the height of the central tables. To compensate for the greater thickness of the ear cartilage, the tables were made of three or four thicknesses of celluloid (Eastman extra-heavy 'kodaloid,' which is 0.62 mm. thick) instead of two. The method of insertion was similar in all respects to that used for the rabbit operations (Clark, et al., '30). In the 'round table' chambers, as already described, the central table, approximately 6.7 mm. in diameter, is inserted in a hole cut clear through the ear and the invasion of new vessels and tissue from the preformed tissue around its periphery, into a thin space of known and controlled thickness, is observed through the mica cover which forms the top of the chamber. The thickness of the space over the table into which the regenerating

tissue and vessels grow is controlled by the thickness of the four small celluloid buffers which are glued to the table and with which the cover is in contact (fig. 1). The space over the tables in the chambers used in these experiments was 72 μ.

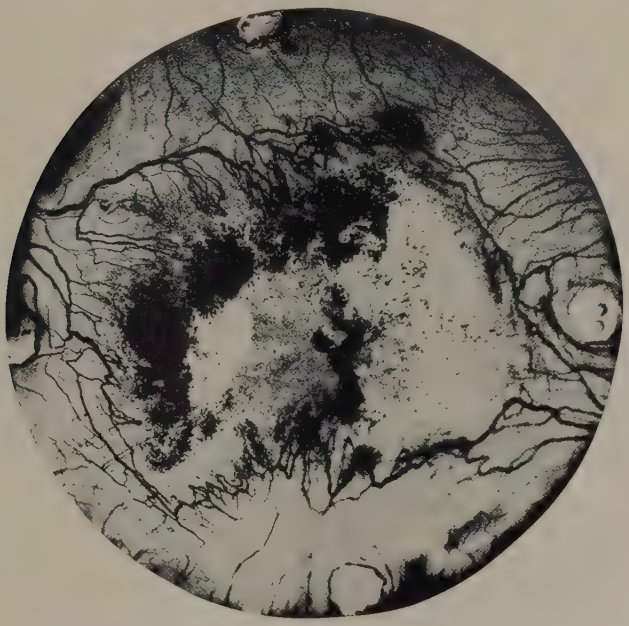

Fig. 1 Photomicrograph of the table area of a chamber inserted in a dog's ear, taken 17 days after operation, during the stage of invasion of new vessels and tissue. First capillaries appeared on the table at its periphery 6 days after operation. Vascularization of table area was complete at 21 days after operation. × 16.5. Photomicrographs made by B. B. Varian.

Altogether four 'round table' chambers have been installed, three in one animal and one in a second. In all four experiments the chamber space over the central tables was invaded from the surrounding tissue by new growths of blood vessels and connective tissue, but with varying degrees of completeness. In the first two chambers the entire table space was never completely filled with tissue because of the instability of

the chambers although there were good growths which could be observed microscopically over periods of 5 and 10 months respectively (figs. 2 and 3). In the two last chambers installed, stability was increased by the use of celluloid and rubber washers around the three bolts used to anchor the chambers

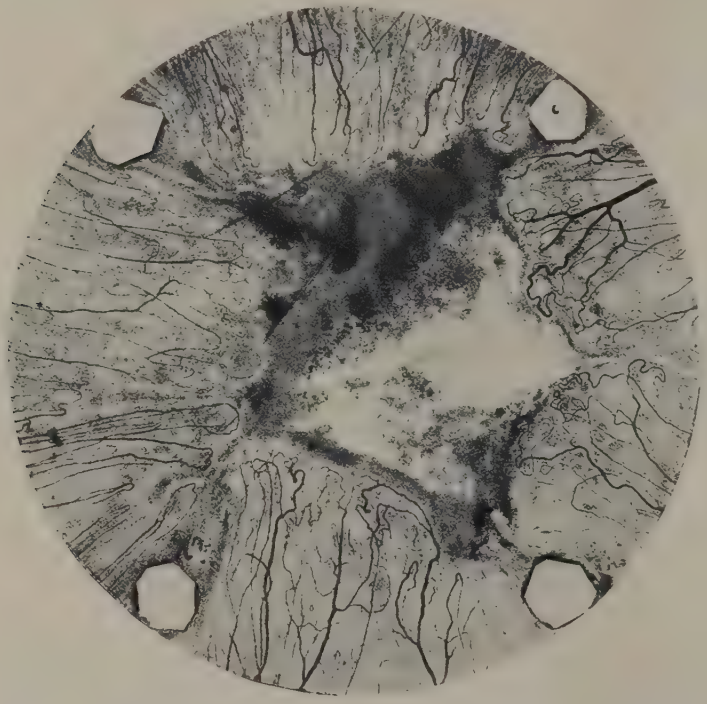

Fig. 2 Photomicrograph of the table area of another chamber taken 17 days after operation. In this chamber the new vessels never reached the center of the table area as a slipping of the new tissue occurred a few days after this picture was taken (p. 394). × 16.5.

in the ear—a return to the technic originally used in the rabbit's ears, as described and illustrated (Clark, et al., '30, p. 199, fig. 3). In both of these the entire table space was filled with new tissue and vessels (figs. 5 to 8). The two chambers remained in the ear for several months. In each case daily

observations of the blood vessels were made for the first month.

The 'preformed tissue' chamber (Clark, et al., '30; Clark and Clark, '32) in which the original vessels of the ear, with their original nerve supply intact, are made available for microscopic study, has also been adapted to the dog's ear and

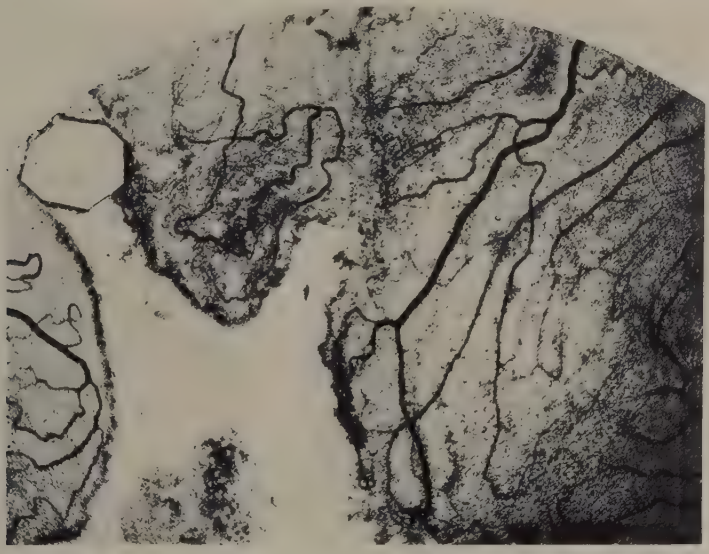

Fig. 3 Higher power photomicrograph of a portion of the same chamber shown in figure 2, taken 30 days after the operation, showing loose character of intervascular tissue. Clear area beyond edge of new vessels and tissue remained fluid in this specimen (p. 292). × 25.

is now being used for studies of the preformed blood vessels in order to compare their behavior with that of the regenerated vessels observed in the round table chambers. The results of these observations will be reported later.

GENERAL OBSERVATIONS

In comparing the conditions found in the round table chambers inserted in the dog's ear with those observed in the

same type of chambers in the rabbit's ear, the first distinct difference noted was in the character and persistence of the exudate which formed around the skin edge which is placed upon the surface of the top and base of the chamber at the time of operation. In the rabbit the exudate persisted for several days, after which it dried and hardened. The skin edge, from the cut margin back to the point where it had been separated from the subcutaneous vascular layer, dried and was sealed tightly to the celluloid surfaces. With the dog, the exudate continued to accumulate in much greater quantity and to remain soft and pliable for many days. Eventually the outer layers dried, the process being accompanied by the formation of large crusts which were removed from time to time. The skin, instead of drying and becoming adherent to the celluloid surfaces of the chamber, remained alive throughout the period in which the chambers remained in the ear.

The second point of difference observed related to the nature of the intercellular substance which formed in the space over the central table area. Observation immediately after the operation for installation of a chamber in the ear of a rabbit showed the presence of fibrin in varying amounts in the space over the table, which is filled with sterile Ringer's solution at the time of operation, while red blood cells, leukocytes and debris were observed moving freely back and forth between the strands of fibrin with each pulse beat and respiratory movement. However, as new tissue invaded the table area from its periphery, the movement of the small particles, etc., ceased, indicating that the fluid had been replaced by or changed into a semi-gelatinous substance (Clark and Clark, '33). In the chambers in the dog's ear, fibrin was conspicuous by its almost complete absence from the growth space. Not more than a few strands have ever been observed, either immediately after the operation or at any subsequent time. In the first two chambers (p. 389) in the dog, the gelatinization of fluid (either inter-cellular or that occuping areas of the central space not yet invaded by new tissue or vessels) did not occur. Instead free fluid persisted over the table continuously,

its presence being shown by the movements of red blood cells, leukocytes, and particles of debris and by the 'washing out' of islands of leukocytes which is described below. In the two more stable chambers, however, a semi-solidification of the intercellular material occurred between the invading blood vessels and also for a distance of approximately a quarter of a millimeter beyond their advancing margin.

The third significant difference observed between the conditions present in the rabbit and the dog concerned the character of the connective tissue which eventually formed in the chambers. In the rabbit all the available space between the vessels covering the table became filled with connective tissue. The formation of connective tissue fibers was much denser in chambers subjected to movement than in the more rigid chambers provided with detached splints (Clark and Clark, '33). In the dog, however, the connective tissue converging toward the center of the table formed only a loose network of fibrous tissue (fig. 3). Erythrocytes and leukocytes became packed in the interstices of the connective tissue fibers thus converting the loose network into a compact mass which was soft and flabby and never became firm as did the tissue in the chambers installed in the rabbit's ear. In the case of the two less stable chambers (p. 392) fluid continued to move among the tissue cells as well as over the central uninvaded portion of the table space. The packed cells—erythrocytes and leukocytes—occupying the intercellular spaces were occasionally washed away by the fluid, leaving larger or smaller denuded areas (fig. 3). Denuding of the larger areas in this manner was usually followed by the collapse of the surrounding tissue or by injury to the blood vessels with haemorrhage. The continual destruction of newly formed tissue, coupled with the tendency of the relatively flabby tissue to slip from the table, explains why the new tissue never completely covered the table area in these first two chambers (fig. 2).

GROWTH AND BEHAVIOR OF BLOOD VESSELS

As has already been stated the growth of new tissue and of vessels into the space over the central table was interfered with in the case of the first two chambers inserted in the dog's ear (p. 393) owing to the slipping of the flabby tissue. In these two chambers, blood capillaries appeared at the periphery of the table at 12 and 13 days, respectively, as outgrowths from preexisting vessels in the surrounding region. The new capillaries grew into the infiltrated tissue, described on page 393, always following the advancing margin of the new tissue. For several days the vessels advanced steadily across the table area, converging toward the center. After this a slipping of the tissue in the chamber occurred and the new tissue, including the newly formed blood vessels, receded at one or more points arount the periphery of the table. In succeeding days the vessels continued to grow and further extension of the vascular network took place but again a slipping of the new tissue occurred and the new vessels and surrounding tissue again receded. Although the new vessels and tissue never completely covered the growth space in these two chambers, circulating vessels remained on portions of the table for as long as 10 months in one specimen, during which time it was always possible to make microscopic observations of the endothelial walls and of the character of the circulation in arterioles, capillaries and venules (figs. 2 and 3).

In the case of the two more stable chambers in which the shifting of the new tissue was prevented in the manner described (p. 390), new capillaries appeared on the table at its periphery at 5 and 6 days respectively and continued to advance steadily thereafter until the vessels from opposite sides anastomosed near the center of the table. Vascularization of the central table space was complete in one chamber at 19 days and in the other at 21 days after operation and at 14 days in one case and 15 in the other after the first appearance of new capillaries at the table edge (figs. 1 and 5 to 8).

The rate of growth of new blood vessels in the two stable chambers averaged 0.5 mm. per day, which is faster than the

average rate of growth for the vessels of the rabbit's ear (0.22 mm.) but the same as that of the more actively growing plexuses of blood vessels in the rabbit (Clark, et al., '31).

In the more stable chambers, in which the denuding of the growth area was prevented, haemorrhages from the tender new capillaries at the growing edge were not so extensive nor so frequent as in those chambers subjected to repeated trauma and it was possible to study microscopic details of their growth. It was found that the new growth of blood vessels took place by the same process of sprouting from preexisting endothelium described for the new capillaries of amphibian larvae and for the regenerating vessels in the chambers inserted in rabbits' ears. In some regions, new capillary sprouts were observed to grow out in advance of the network of connective tissue in the same manner occasionally observed in the rabbit (Clark and Clark, '33, p. 297). As was the case in the chambers in the rabbit's ear, the process of growth of new blood vessels across the table space was accompanied by a remodelling of the older portions of the new vascular network into a relatively stable 'adult' pattern. This remodelling process was accomplished in the same manner as in the rabbit by differentiation of certain of the newly formed capillaries more favorably situated as to supply or drainage into new arterioles or venules and by the reduction in the number of capillaries through retraction of endothelium (Sandison, '28; Clark, et al., '31).

The most striking vascular difference noted in the microscopic study of the circulating blood vessels in the dog, as compared with those of the rabbit, was the rapidity of the circulation in many of the smaller vessels. Unlike the very volatile flow—now rapid, now sluggish, and showing frequent reversals of direction—that is characteristic of capillary circulation in the rabbit (Sandison, '32), the movement of blood cells through a great many of the smaller vessels in the dog was seen to be extremely rapid and constant.

Careful measurement of a large number of blood vessels at the narrowest point between arteriole and venule showed that

their diameter varied from 13 μ to 34 μ with an average of slightly more than 17 μ. This is wider than the diameter of typical capillaries of the vascular networks which formed over the table area in chambers in the rabbit's ear, i.e., 8 μ, to 12.6 μ (Clark and Clark, '34 b). The situation of these connecting vessels in the dog, between the arterioles and venules and the single layer of endothelium composing their walls suggested capillaries but the rapidity of their blood flow and

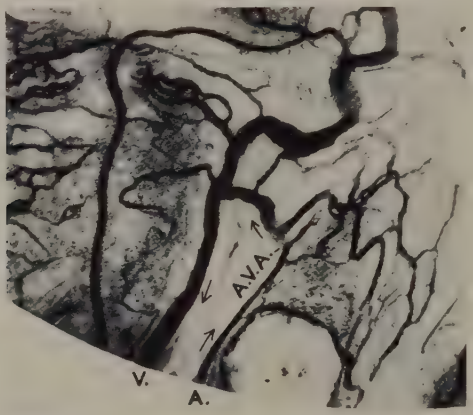

Fig. 4 Photomicrograph of a small region of the same chamber illustrated in figures 5 to 8. Shows character of blood vessel network. The type of circulation indicated by arrow suggests an arterio-venous anastomosis A.V.A. A., artery; V, vein. × 32.

their relatively large diameters indicated an arterio-venous type of circulation. Figure 4 is a photograph of such a connecting vessel which was observed intensively for several weeks. Although such relatively wide connecting capillaries with rapid blood flow persisted for weeks, none of them was observed to acquire the thick layer of muscle cells and active contractility observed in both the preformed and new-formed vascular networks in the rabbit's ear (Clark and Clark, '34 a and b).

Among the numerous studies already carried out in the transparent chambers in the rabbit's ear have been extensive observations on the behavior of small blood vessels in which it was found that in contrast to the condition in Amphibia in which vascular endothelium is definitely contractile (Clark and Clark, '25) the capillaries and venules of the rabbit show only passive changes in caliber and that active vascular contractility is confined to arteries, arterioles and arterio-venous anastomoses and to a few large veins, i.e., to vessels with smooth muscle cells on their walls (Clark and Clark, '32, '34; Sandison, '32). This was true of both regenerated vessels in the various types of new growth chambers and of the original vessels of the ear studied in 'preformed tissue' chambers. It was further shown that in the case of regenerated vessels, the newly formed arterioles with muscular walls do not acquire the power of active contractility unless or until they receive a new nerve supply (Clark, Clark and Williams, '34). The many investigators who have reported capillary contractility in mammals have made their studies for the most part in regions which were not sufficiently transparent to enable them to see the walls of the smaller vessels distinctly and hence they could not distinguish between arterioles, which are frequently narrower than capillaries, and true capillaries and could not determine whether vessels which were empty of blood were actually contracted, contained plasma alone, or were compressed. These studies on the dog were begun with the recognition that the difference found in the contractile power of endothelium in the rabbit from that present in Amphibia might not be typical for mammalian vessels in general.

Long continued observations of individual capillaries have been made in all of the transparent chambers inserted in the dog's ear. In one instance a single capillary, which was observed to be formed by the anastomosis of two capillary sprouts, was studied every day for nearly a month. When observation was begun there were no adventitial cells on either of the sprouts, but they made their appearance later on the wall of the capillary formed by the anastomosis and remained

on the wall throughout the period of observation. No active contraction of this capillary was seen either before or after the appearance of adventitial cells outside its endothelium. Many other capillaries, some of them already supplied with adventitial cells when observations were begun and some lacking them, have been studied daily for long periods and in no instance have active contractions been observed. From these preliminary observations on the regenerated blood vessels in the dog's ear the same results as regards the lack of active contractility of capillaries were obtained as from the numerous observations on both the preformed and new formed vessels in the rabbit's ear (Clark and Clark, '32, '34; Sandison, '32).

In contrast to the failure to find any evidence of capillary contractility, active contractions of both the spontaneous variety as well as those elicited by tactile or auditory stimulation of the animal, have been observed in the arteries and arterioles of the dog's ear. Thus, according to observations carried out up to this time, active vascular contractility in the dog, as in the rabbit, appears to be confined to vessels with smooth muscle on their walls. However, in the dog, the spontaneous arterial contractions were less marked and much less frequent than was the case in the rabbit vessels, while a much greater stimulus was necessary to induce a generalized arterial contraction. Gentle prodding of the animal with the finger, which produced such a constant response on the part of the arteries of the rabbit's ear had no effect on similar vessels in the dog. Actual pinching of the animal or a sudden loud noise, such as slamming of a door, were necessary to induce general contraction of the arteries and arterioles. Perhaps a part of this difference in arterial response may have been due to the great docility of the type of dog used in these experiments since individual differences in arterial contractility were observed in different rabbits corresponding to differences in temperament (Clark and Clark, '32).

At present studies are in progress on the vessels of the dog's ear as observed in 'preformed tissue' chambers, where

the original ear vessels with intact nerve supply can be observed and their reaction to various drugs studied, in which it is hoped that further information as to the contractility of the blood vessels of the dog can be obtained and compared with the results of similar studies already carried out on the vessels of the rabbit.

SUMMARY

The transparent chamber for the microscopic study of living cells and tissues in the living mammal has been successfully adapted for insertion in the dog's ear. This makes it possible to carry out in another mammal the same type of prolonged microscopic study which has been accomplished in the transparent chambers inserted in the ears of rabbits.

The 'round table' chamber designed for the study of new growth of blood vessels and other tissues was used for the series of experiments reported at this time. Microscopic observations of the same vascular areas in four such chambers were carried out for periods of 5 to 10 months. A 'preformed tissue' chamber for the study of the original blood vessels of the ear has also been successfully installed and the results of observations made in this type of chamber will be described later.

The type of dog (beagle hound) used for these experiments proved to be well adapted, both in regard to the size of its ears and its docility, for the installation of permanent chambers and for prolonged microscopic observations.

Observations on some phases of the growth and behavior of the new tissues which invaded the thin transparent observation space in these chambers have shown the following similarities and differences in comparison with the rabbit:

The exudate which formed around the edges of the chambers persisted longer in the dog than in the rabbit and remained softer. Within the chamber space the fibrin which formed following the operation was much less in amount in the dog. The consistency of the intercellular substance which formed between the new blood vessels which invaded the table area was softer and more flabby in the dog than in the rabbit.

After the table space had been completely covered by new blood vessels and tissue, the new connective tissue fibers formed a much looser network in the dog than was the case in the chambers in the rabbit.

The growth of new blood vessels into the space over the table area took place in the same manner in the dog as in the rabbit, by the sending out of capillary sprouts from pre-existing vascular endothelium and by the anastomosis of neighboring sprouts to form a plexus. Differentiation of new arteries and veins from the indifferent capillary plexus also occurred in the same manner observed in the rabbit. The rate of growth of new capillary endothelium was the same as in the more rapidly growing vessels in the rabbit.

The circulation through the vessels of the table area in the chambers inserted in the dog's ear was more rapid and steady than was the case in the rabbit. In a number of cases the rapid flow of blood through relatively wide connecting vessels between arteries and veins resembled the arterio-venous type of circulation common in the vessels of the rabbit's ear but the thick muscular walls characteristic of both the preformed and new-formed arterio-venous anastomoses in the rabbit were absent in the dog.

Prolonged microscopic observation of many individual capillaries both before and after their acquisition of adventitial cells failed to show any evidence of capillary contractility. The active contractions of blood vessels seen in the round table chambers inserted in the dog's ear were limited to arteries and arterioles—to vessels with smooth muscle on their walls—as was the case in the vessels of the rabbit. The active spontaneous contractions of arteries in the dog's ear were found to be much less frequent than in similar vessels in the rabbit and much greater external stimulations of the animal were required to produce generalized contraction of the arteries in the dog than was the case in the rabbit.

Further studies of the original blood vessels of the dog's ear in 'preformed tissue' chambers will be necessary before it will be possible to state definitely that the active contrac-

tions of blood vascular endothelium, observed in the capillaries of Amphibia, are absent in the dog as well as in the rabbit. Such studies are now in progress.

This work was undertaken at the suggestion and under the direction of Dr. Eliot R. Clark, to whom the author wishes to express his thanks.

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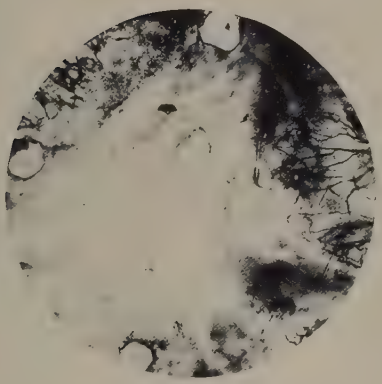
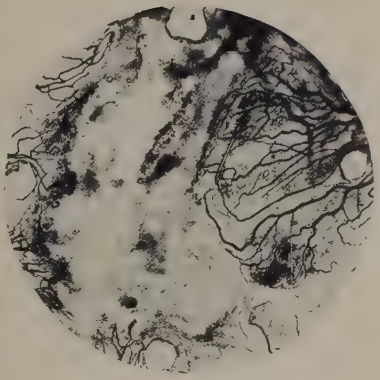
PLATE 1

EXPLANATION OF FIGURES

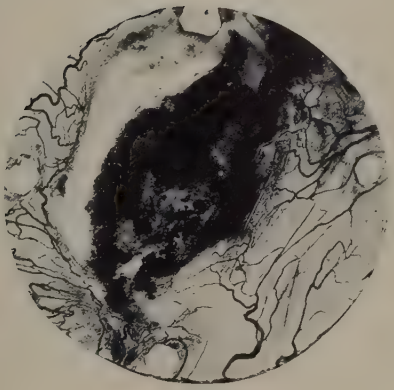
5 to 8 Series of photomicrographs showing the growth of new vessels and tissues on the central table of a chamber inserted in the ear of a dog. New vessels invaded the space on the table, at the periphery, 5 days after installation of the chamber. Vascularization of table area was complete at 19 days after the operation (fig. 8). Extravasated blood (dark area in photo) which increased in amount at 13 days (fig. 7) owing to haemorrhage from the delicate tips of the new capillaries, was gradually absorbed. The small amount represented by the small dark patch to left of center in figure 8, was completely absorbed 2 days after anastomosis of vessels from opposite sides of chamber. × 9.6.



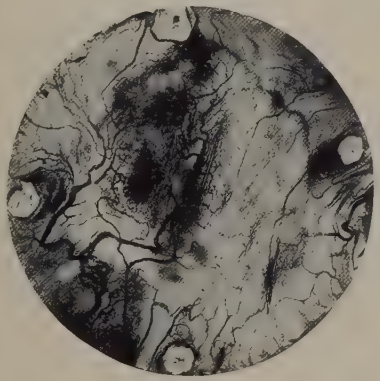
5
(6 days)



6
(10 days)



7
(13 days)



8
(19 days)

THE ORIGIN OF THE NOTOCHORD IN ALLIGATOR MISSISSIPPIENSIS

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SIX FIGURES

The origin of the notochord is variously described by different writers, some deriving it from the entoderm, some from mesoderm, and some from the undifferentiated cells in the region of the blastopore.

In the American alligator, so far as could be determined with the material at hand, the writer is inclined to the opinion that the notochord is of entodermal origin. This material, consisting of about a half dozen series of sections of early stages of development, is of some historic interest. In the Journal of Morphology, vol. 5, 1891, was published, by Dr. Samuel F. Clarke, one of the first researches dealing with the American alligator. This paper, "The habits and embryology of the American alligator" gave, with excellent figures that have been much copied, a brief account of the embryology of this well-known reptile. The present writer, in spite of two trips to Central Florida, one to the Okefenokee Swamp and one to South America, never succeeded in securing the very early stages in development. In fact, to the present day, so far as he is aware, the segmentation and preceding processes have not been studied.

During Doctor Clarke's life he was most generous in allowing the writer to examine his material and after his death his widow kindly made the writer a gift of his entire collection of alligator slides. These slides, from which the accompanying drawings were made are, in some cases, dated 1889, 46 years ago, and are still in almost perfect condition.

Some of the sections are rather pale, whether because of the original staining or on account of fading it is not possible to say.

Those of us who were privileged to know Doctor Clarke still miss the quiet, handsome, courteous old gentleman that we were accustomed to see at scientific gatherings; and the writer values, for sentimental as well as scientific reasons, the slides that Doctor Clarke made so many years ago.

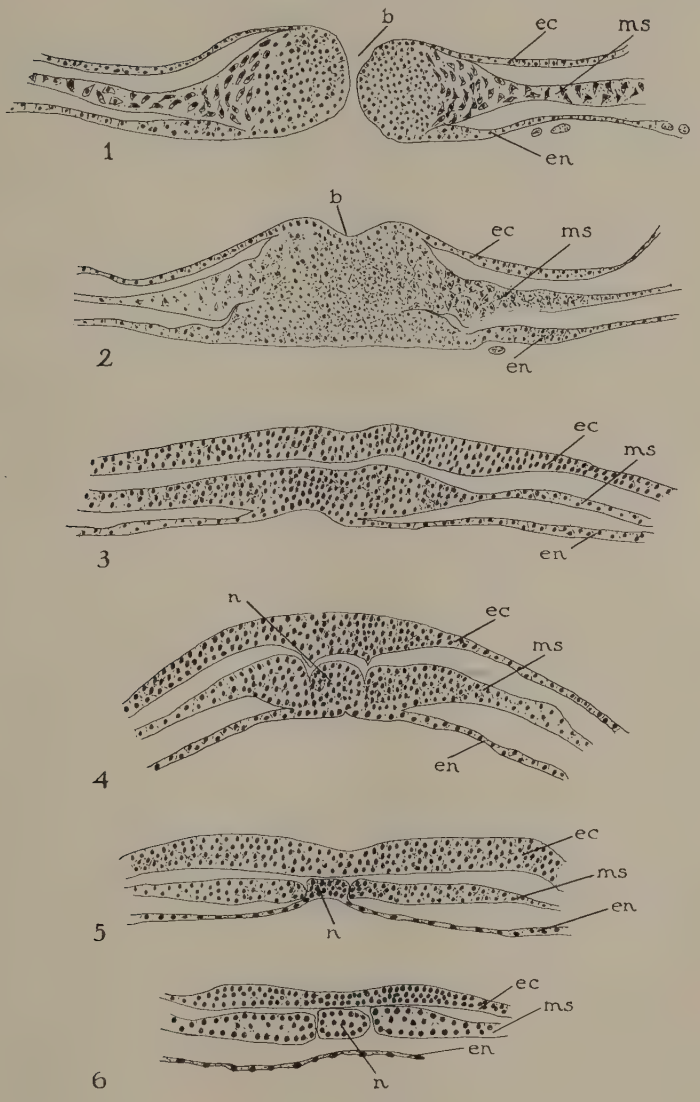
Figures 1 to 6 represent transverse sections through very early embryos, all of approximately the same stage of development. They were all outlined with a camera lucida under the 16 mm. objective and 10 × ocular.

Figure 1 represents a section passing directly through the blastopore (b), a narrow but distinct opening passing through the blastoderm at a fairly acute angle. The ectoderm (ec) and entoderm (en) are here of about the same thickness, and they fuse insensibly with the large mass of mesoderm (ms) at the margin of the blastopore.

Figure 2 represents a section through the anterior margin of the blastopore (b) a short distance in front of the preceding section. The ectoderm, entoderm and mesoderm are still fused to form a large, compact mass of cells in the median plane. The ectoderm and entoderm are still of the same approximate thickness.

Figure 3 is a few sections anterior to the preceding. The ectoderm, which is here much thicker than the entoderm, is entirely distinct from the other layers but shows no indication of medullary folds, in this posterior region. The entoderm which is here very thin, is quite indistinguishable from the mesoderm in the median region.

Figure 4, a few sections anterior to the last, shows the thin entoderm and the thick mesoderm still fused mesially; but in the center, the notochord (*n*), is beginning to differentiate as a circular or rectangular mass. In this section it would be impossible to say whether the notochord were the medial region of the mesodermic sheet or a thickening from the dorsal side of the entoderm.



Figures 1 to 6

Figure 5, a very short distance anterior to figure 4, shows the notochord entirely separated from the mesoderm on each side, but still indistinguishably fused with the entoderm below. This particular section would seem to indicate that the notochord is derived from the entoderm, unless it had previously been a distinct rod of cells and had secondarily and temporarily fused with the entoderm. There is nothing to indicate such a fusion in the material studied.

Figure 6, a section cut at some distance anterior to the last, shows the notochord quite distinct from the mesoderm on each side and the entoderm beneath. There is nothing here, of course, to indicate from which germ layer the notochord may have been developed. The great difference in the relative thickness of ectoderm and entoderm is shown in all of the sections except figures 1 and 2.

AN EXAMPLE OF THORACOPAGUS TRIBRACHIUS IN THE MOUSE

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TWO FIGURES

Although teratological individuals appear occasionally among most stocks of mice, certain types are much more rare than others. Probably the most frequent form manifests the absence or reduction of the lower jaw, agnathia, with accompanying distortion of the facial region. Little and Bagg ('24) have described and depicted mice of this classification, determining that in some instances at least its appearance has a definite hereditary basis. Morphologically, it resembles some of the lower grades of otocephaly reported by Wright ('34) in the guinea pig. Sporadically, however, otocephalic monsters arise in other strains of mice, one example occurring in our stock of Mus bactrianus.

A more marked deviation from normality, structurally, is found in the 'posterior duplication' mice reported by Danforth ('30). The most extreme mice of this strain are nonviable monsters with ectopic viscera exhibiting a complete duplication of the pelvic region and its appendages. The tendency for posterior doubling is clearly hereditary in the strain although the great majority of animals are phenotypically normal. Since still more abnormal monsters seem uncommon in mice it may be of interest to record an example of thoracopagus, the only one of its kind found among the half million or more mice observed by workers at this laboratory.

This monster was one of the six stillborn in a litter of nine—all the others being normal—borne by an inbred *dba* female

mated to an F_1 of *dba* and brachyury (*Tt*) ancestry. When found, the top of the head of the thoracopagus above the level of the ears and eyes had apparently been eaten away by the mother although it is possible that part of the head abnormality was occasioned by cranioschisis. Since mice often eat

Fig. 1 External view of thoracopagus tribrachius.

their stillborn and abnormal offspring it may be that some of the extreme monsters are thus disposed of.

Each half of the doubled body was somewhat smaller than usual in newly born mice. Figure 1, presenting a photograph of the preserved specimen, shows that both were females, judging by the external genitalia, and that both were phenotypically normal tailed (tt).

Figure 2, a photograph of the mouse cleared and stained with alizarin red S, shows details of the skeleton. Despite the single thoracic cavity of the thoracopagus, it possesses two complete vertebral columns, each with its normal complement

Fig. 2 Skeleton of thoracopagus tribrachius.

of ribs. There are four normal posterior appendages and but three anterior. One of the latter has two scapulae with but a single humerus, perhaps representing the result of embryonic fusion.

The two sterna are present is an atypical position. One lies between the right ribs of one body and the left ribs of the second while the other is situated between the left of the former and the right of the latter. The posterior parts of the skull show indications of doubling but the anterior portion of the head is completely normal with two nasal bones, one lower jaw, two zygomatic arches, two eyes and two ears. The rostrum, however, appears short and underdeveloped for a full term fetus.

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NEW BOOKS AND PUBLICATIONS

LACTOBACILLUS ACIDOPHILUS AND ITS THERAPEUTIC APPLICATION, by Leo F. Rettger, Maurice N. Levy, Louis Weinstein and James E. Weiss. 1935. Size, $5\frac{1}{2} \times 8\frac{1}{4}$ inches. Pages vi + 203. Bibliography. Cloth. Price, $2.50. Yale University Press, New Haven.

This volume is a report of experiments investigating the influence exerted by different carbohydrates, particularly lactose and dextrin, and by milk, on the character and numerical relations of the intestinal bacteria of man and the lower animals; and the establishing of a predominantly aciduric flora by the oral administration of cultures of Lactobacillus acidophilus. It describes Lactobacillus bifidus and other oral and dental types of lactobacilli and their relation to Lactobacillus acidophilus. Factors affecting the viability of Acidophilus and its successful therapeutic application to cases of simple constipation and constipation accompanied by other symptoms are discussed. Numerous case histories are given and the results summarized in a general discussion. An appendix gives brief descriptions of media and methods.

HANDBUCH DER ANATOMIE DES KINDES, edited by Karl Peter, Georg Wetzel and Friedrich Heiderich. Zweiter Band, 4 Lieferung. 1936. Size, $6\frac{3}{4} \times 10$ inches. Pages 729 + 844. 70 illustrations. Price, 27 RM. Julius Springer, Berlin.

This comprehensive treatise on the anatomy of the child is being published in sections. The present issue is the fourth part of the second volume. It describes the membranes of the brain and spinal cord, and the glands of internal secretion.

LES ARTÈRES MÉSENTÉRIQUES, by A. Hovelacque. 1936. Size, $7\frac{1}{4} \times 10\frac{1}{2}$ inches. 112 pages. 35 illustrations by A. Moreaux. Bibliography. Price, 30 francs. Gaston Doin et Cie, Paris.

Professional men engaged in abdominal surgery, as well as students of anatomy, will appreciate the usefulness of this volume on the mesenteric arteries. Text and illustrations picture accurately the origin, course and endings of these arteries and their relations to veins, nerves, lymphatics and visceral organs. The bibliography is extensive.

A TERMINOLOGY OF OPERATIONS OF THE UNIVERSITY OF CHICAGO CLINICS, by Hilger Perry Jenkins. 1935. Size, $5\frac{1}{4} \times 7\frac{1}{2}$ inches. Pages xx + 99. Index. Price, $1.00. The University of Chicago Press, Chicago.

The terminology of operations as set forth in this volume can do much to eliminate the difficulties experienced in looking up operations of a certain type or in compiling statistical data on such operations. Individual surgeons have

different ways of wording their reports and different names for the same operation. In any previous system of filing records, identical procedures might be widely separated under several names, all accepted as correct. The need for standardization has long been apparent. This small volume is designed for the record librarian. It gives instructions for setting up and maintaining the filing cabinet and for using the index. The chapters of the book take up operations on different regions, systems and organs: tegumentary and connective tissue structures, glandular and vascular systems, abdominal, orthopedic, thoracic, neurological, oral, oto-rhino-laryngological, ophthalmological, urological and gynecological surgery, obstetric operations and radium therapy. All synonyms are grouped together. The name chosen to head the list is the most correct form in common use, preferably one of anatomical derivation. The index lists alphabetically all terms and synonyms appearing in the book. Intelligent application of this system in hospital filing would prevent the disappearance of valuable records behind some correct but generally unrecognized title.

A CYTOLOGICAL STUDY OF THE DIGESTIVE SYSTEM OF ANURAN LARVAE DURING ACCELERATED METAMORPHOSIS

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TWO HELIOTYPE PLATES (THIRTY-FIVE FIGURES)

INTRODUCTION

Since the report by Gudernatsch ('12), that the feeding of thyroid tissue accelerates metamorphosis of some amphibia, a great many physiological and morphological investigations have been made on precociously metamorphosed forms. Studies of morphological changes in accelerated, as well as normally metamorphosed amphibia, have been confined chiefly to macroscopical and general histological changes. The reports of Reuter ('00 a), Yung ('04), Bowers ('09), Swingle ('18), and Kuntz ('24) give in some detail the macroscopical changes in the anuran alimentary tract as evidenced by a decrease in weight and linear measurement. Ratner (1891), Reuter ('00 b), Deusberg ('06), Kuntz ('24), and recently Janes ('32, '34) have reported at considerable length on the histological changes occurring in the digestive tract during metamorphosis.

Very little, if any, detailed cytological study of the changes which take place during metamorphosis is available at present. This work attempts, in part at least, to give this information for the digestive organs of the bullfrog, Rana catesbiana, in some stages during its thyroid accelerated transformation from the limbless tailed larval form to the limbed tailless adult.

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MATERIAL AND METHODS

R. catesbiana larvae were used in this study. All the animals were in the late larval, pre-metamorphic stage, some few showing the slightest differentiation of hind limb buds, with no other signs of metamorphosis. The cytological studies were confined to the epithelial and gastric gland cells of the stomach, the epithelial cells of the intestine, the acinal cells of the pancreas, and the hepatic cells of the liver.

The cytological studies were correlated with external changes during the metamorphic period. A series of six stages, as shown below, based on the appearance of limbs, were chosen in making this study:

Stage I (tadpole stage). This is the normal, untreated, pre-metamorphic tadpole, with or without the slightest indication of hind limb buds.

Stage II (hind limb stage). The hinds limbs are small but well differentiated.

Stage III (opercular stage). The hind limbs are increased in size. The fore limbs are well differentiated in the opercular sacs.

Stage IV (left limb stage). The left fore limb has just broken out. In all cases noted here the left fore limb emerged first.

Stage V (right limb stage). The left fore limb is well out. The wall of the right operculum had degenerated, leaving a small opening, and the folded right fore limb is visible within the opercular sac.

Stage VI (final stage). Both fore limbs are well emerged and differentiated.

For routine studies, tissues were fixed in either Zenker's or Bouin fluid and stained with hematoxylin-eosin. The Champy-Kull method, with acid fuchsin and toluidin blue as stains, was used to demonstrate mitochondria. The Nassonov

modification of Koletschov's technique was used to demonstrate the Golgi apparatus. Some of these sections were left unstained for the study of Golgi substance others were stained with aqueous acid fuchsin to bring out the mitochondria. All sections were studied at 5 μ. Impregnation with a 2 per cent osmic acid solution was carried out at 35°C. It was found necessary to allow a longer impregnation time for the later stages. Eight days of impregnation were sufficient to bring out the Golgi apparatus in stage I; 14 days were necessary for stages II, III, and IV, and 21 days for stages V and VI. In spite of this, results obtained for stage VI could not be considered satisfactory, impregnations being quite unevenly distributed throughout the tissues. A longer impregnation time would be quite precarious because even at 21 days the tissues become very brittle and difficult to handle.

Thyroxine was used to accelerate metamorphosis. Pure thyroxine crystals were dissolved in a small quantity of NaOH, and then diluted with distilled water to make a stock solution of 100 cc. The animals were treated every other day in a solution of thyroxine: tap water, in concentration of 1:600,000. During treatment, not more than three animals were kept in one glass container. Three liters of solution were used per treatment. On days alternating with thyroxine treatment, the animals were kept in tap water and were offered food. With such treatment, both fore limbs emerged in about 15 days.

OBSERVATIONS

A. Stomach

1. Epithelial cells. All cells of the epithelium and its glands are not lost during metamorphosis. In general, some are retained and differentiate into definite mucosal and glandular cells. In describing earlier stages, the most representative cells of a given stage are chosen, because it is some of these cells which in all probability regenerate to form the definite tissues. This same principle applies to the descriptions of cells studied in the other organs.

The epithelial cell of the tadpole's stomach (stage I) is a ciliated, elongate columnar cell. The flattened oval nucleus is usually centrally located, dividing the cell into an apical or thecal region, toward the lumen, and a much narrower basal portion. The finely granular cytoplasm stains intensely with eosin, except at the apical border just below the cilia where a somewhat lighter area occurs (fig. 3). The nucleus contains a heavily staining chromatic reticulum. Large chromatic masses prevent the sharp distinction of nucleoli. The cells normally secrete by bursting the apical cell wall. The basal cells are somewhat rounded, with large nuclei filling the greater part of the cells. The cytoplasm of these basal cells is also finely granular and stains well with eosin.

The first indication of a change (stage II) is the accumulation of masses in the cytoplasm toward the free border of the columnar cells. These masses stain deeply with hematoxylin. This is soon followed by a loss of cilia. However, the persistence of cilia in some isolated cells has been noted even through stage IV. Following the loss of cilia, a bursting of the whole apical region of the cell occurs, and the darkly staining masses, together with a portion of the cytoplasm, are cast off. The retained cytoplasm at this stage takes a lighter eosin stain. With the bursting of the theca the whole cell seems to contract, and soon (stage III) the cells walls become indistinct and the epithelium is syncytial in nature. The nuclei become more rounded, and the former columnar cells are now scarcely distinguishable from the basal cells below; they can be identified by means of position only. The cytoplasm contains small, clear droplets which give it a reticular appearance. The nuclei are still round or oval, and present a course, chromatic reticulum. In some few places, however, in stage IV, we again begin to see the appearance of cell walls, and in stage V the cell walls have entirely reappeared. In this stage we get the first significant indication of mitosis in the epithelium. This occurs only in the lower cells of the epithelium, corresponding to the position originally held by the basal cells.

Following the establishment of cell walls, and probably due to the increased number of cells which results in crowding, the cells become elongate again, so that in the final stage (VI), most cells are once more tall columnar. The well-known general contraction of the whole organ may also play a part in this, but since most of this contraction occurs in the earlier stages of metamorphosis, it can therefore play but a minor role in the elongation of the epithelial cells. The cytoplasm (stage VI) stains well with eosin, is finely granular, and may contain a few non-staining droplets. The apical border of the columnar cell is characterized by a clear, non-staining area, apparently filled with mucus (fig. 4). The nucleus is again somewhat flattened and shows a coarse, slightly chromatic reticulum. Generally one or two distinct nucleoli may be found in the epithelial cells.

The mitochondria of the epithelial cells, as well as of all the other tissue described, are easily demonstrated in Champy-Kull preparations. These, however, are all confirmed in Golgi preparations counter-stained with aqueous acid fuchsin. The columnar cell of the tadpole stage (I) shows an even distribution of short, curved or rod-like mitochondria scattered throughout the cytoplasm (fig. 2). These stain dark blue in the Champy-Kull preparations, and light pink in Golgi preparations counter-stained with aqueous acid fuchsin. With the bursting of the theca in stage II, some of the mitochondria are probably lost with the cytoplasm because the concentration in the syncytial state (stages III, IV) shows the same general distribution as in stage I. With the reestablishment of the cell wall and the elongation of the cell in the final stage (VI) the same general distribution is found (fig. 1). No quantitative or qualitative change in the mitochondria can be discerned between stages I and VI.

One of the most interesting findings in the course of this investigation was the behavior of the Golgi apparatus. Golgi descriptions for all tissues follow from unstained Nassanov-Koletschov preparations. The columnar cell (stage I) shows a distinct, black staining Golgi reticulum which is concentrated

chiefly above the nucleus, and may extend up in the cytoplasm as much as halfway toward the apex (fig. 5). A much lighter reticular band is found extending downward around the nucleus, somewhat in the form of a cap, and often the whole nucleus is surrounded. The basal cell in this stage shows the Golgi as a dense reticulum completely surrounding the nucleus.

With the onset of metamorphosis (stage II) we find the Golgi apparatus undergoing distinct changes. Even before the loss of cilia, the distinct Golgi reticulum begins to fragment and spread through the cytoplasm. After the loss of cilia, and at the time of the bursting of the theca, the Golgi substance is found to have assumed a granular appearance. The granules are generally distributed through the cytoplasm, but are more concentrated about the nucleus (fig. 6). With the bursting of the theca most of these granules are lost (stages II, III). Simultaneously with these changes in the columnar cells, the Golgi apparatus of the basal cells also fragments and show a similar distribution of granules in the cytoplasm, with a greater concentration around the nuclei. Toward the end of the syncytial state (stage IV), most of the granules of the general cytoplasm have disappeared, leaving only those concentrated about the nuclei. These granules apparently condense to form a reticulum, because most of the cells in this stage (IV) show a very thin reticular Golgi layer, completely surrounding the nuclei of the retained and regenerated columnar and basal cells (fig. 7). Occasional osmiophilic droplets are seen in the cytoplasm.

With the reformation of the cell walls there is little change in the structure of the Golgi substance, except that the reticulum appears more conspicuous (stage V). In the final stage (VI) the original reticular nature of the Golgi substance is reestablished. The network is seen completely surrounding the nucleus of the columnar cell (fig. 8) and the basal cell. It is, however, much reduced in comparison to the condition found in the tadpole stage (I). The conspicuous network found above the nucleus in the columnar cell of stage I, is almost completely absent here, although in an occasional cell

a few strands of Golgi network can be seen extending above the general reticulum surrounding the nucleus. A similar behavior of the Golgi apparatus is found in the other tissues studied.

2. Gastric gland cells. The gland cell of the tadpole stomach (stage I) has a large, generally round nucleus which is usually basally located. The nucleus is lightly reticular, and shows chromatic masses which stain darkly with hematoxylin and which cannot be distinguished from nucleoli. The cytoplasm is intensely stained with eosin, and shows some coarser, homogeneously distributed granules which take the hematoxylin stain (fig. 9).

The first apparent change in these cells (stage II) is a clumping of these dark granules. Thus, dark masses are seen in the cytoplasm, and these are found in general more in the region bordering the lumen. The cell in general appears somewhat contracted, and the cytoplasm vacuolated. The cell wall is less distinct, and soon, in this and in the following stage, the dark masses are no longer visible. Thus in the opercular stage (III) a general syncytial condition is found. It is, as yet, uncertain whether the dark masses referred to are ejected into the lumen. If they are, they must lose their staining reaction in the process. The retained cytoplasm is finely granular and shows some few non-staining droplets; the dark masses are no longer present. An occasional mitosis is seen.

Practically the same picture is visible in the left limb stage (IV), except that a greater number of mitoses occur, and some cell walls again become visible. This continues into the next stage (V), where the cell walls are again seen in all cells. In this stage cell division is still progressing at a rapid rate. In the final stage (VI) the glands are again reformed and all the cells are distinct. The nuclei are generally round, and show a faint reticular background with one or two distinct nucleoli. The cytoplasm is finely granular and the coarse granules of stage I are absent in these gastric gland cells.

The mitochondria in these gland cells of the tadpole in stage I are short, curved or rod-like forms, found abundantly

scattered throughout the cytoplasm (fig. 11). No apparent change is seen in the mitochondria in stage II, but with the appearance of the syncytial state (stage III), the mitochondria are found to have greatly decreased in number; only few scattered bodies are visible in the cytoplasm. This condition persists until the formation of the cell walls in stage V. There then appears to be a slight increase in the number of mitochondria over the previous two stages, but there are definitely less present than in stage I. This condition persists into the final stage (fig. 12). There is a definite quantitative difference in mitochondria between stages I and VI, which is evidenced by a decrease in number in the final stage (VI). This decrease is confirmed in Golgi preparations, counterstained for mitochondria.

The Golgi apparatus found in the gastric gland cells in stage I, is in the form of a heavy reticulum completely surrounding the nucleus (fig. 13). In the following stage (II), the reticulum fragments, as in the epithelium, and becomes granular (fig. 14). In the syncytial stage (III) this granular state is still apparent, and it is seen as a loose, granular ring, completely surrounding the nucleus. In stage IV, the Golgi substance is again seen as a much reduced, thin compact reticulum completely surrounding the nucleus. This condition persists to the formation of the definitive cell, and in stages V and VI the Golgi substance is present as a distinct, compact reticulum, completely surrounding the nucleus, but somewhat more conspicuous than in stage IV. It is, however, hardly as dense as in the tadpole stage (I). There has resulted a definite decrease in the amount of demonstrable Golgi substance (fig. 16).

B. Small intestine

The chief cell in the intestine of the tadpole stage (I) is a ciliated, tall columnar cell. The nucleus is usually centrally located and shows a fine reticular background in which are set dark staining chromatic clumps (fig. 17). The finely granular cytoplasm stains well with eosin. Small non-staining

droplets can be seen in the distal cytoplasm of some cells. In other cells these small droplets can be seen coalesced into larger drops, which apparently make their way through the cytoplasm toward the apical border of the cell, thus giving rise to a goblet cell. This goblet is filled with a non-staining fluid, and is often quite large, filling the greater part of the cell above the nucleus. After secretion, in which the contents of the goblet are lost, the thecal portion of the cell is found collapsed, and the cell presumably is returning to its initial chief cell stage. The basal cell in stage I of the intestine is a round cell lying just above the basement membrane. The nucleus is round, fills a greater portion of the cell, and shows a coarse, chromatic reticulum. The cytoplasm is finely granular and stains well with eosin. An occasional mitotic figure is seen in these cells. Transitional forms may be observed in which the round basal cells elongate and force their way toward the periphery between the columnar cells. These forms give rise to chief columnar cells.

With the onset of metamorphosis (stage II) there is a great increase in the number of goblet cells. Observations indicate that the chief cells pass through the goblet state previous to the loss of cilia and general collapse of the thecal portion of the cell. Some areas of the epithelium in this stage (II) have already lost their definite columnar character. The cytoplasm is finely granular and stains less intensely with eosin. The reticuli of the nuclei are somewhat more chromatic. Occasional mitotic figures are present in the area of the basal cells.

In the opercular stage (III), no goblet cells are visible and the entire retained epithelium is syncytial. The cytoplasm is much reduced and contains some few, small, non-staining droplets. The nuclei of the retained columnar cells are more rounded than in stage I, and are, in most cases, indistinguishable from the deeper lying nuclei of basal cells. Mitoses are now especially abundant in the nuclei lying in the more basal portion of the syncytium. In all probability regeneration is confined entirely to the retained basal cells. There is little

change in stage IV, except for an increase in mitosis and the reappearance of some cell walls. This process continues into stage V, wherein, apparently due largely to the increase in cell number and also to the general contraction of the intestine, the cells again assume their columnar nature. The nuclei in these tall cells are flattened. The cytoplasm stains well with eosin, and in many cells small droplets can be seen above the nuclei. A few small goblet cells also appear. This continues on into stage VI in which there appears to be a slight increase in the number of these cells. The goblet cells, however, are few in comparison to the chief cells. The definite chief cells (figs. 19, 22) and goblet cells are tall columnar and non-ciliated with the nucleus centrally located. The cytoplasm is filled with fine granules and may contain various amounts of small, non-staining coalescing droplets. Round basal cells, some of which may be undergoing division, are found lying above the reformed basement membrane.

In the intestine (stage I), the mitochondria in the chief cells (fig. 18) and goblet cells are short, curved or rod-like bodies staining intensely blue-black in Champy-Kull preparations, and light pink in Golgi preparations counter-stained with aqueous acid fuchsin. These cytoplasmic inclusions are found scattered throughout the cytoplasm. In the goblet cells the mitochondria are slightly more concentrated around the goblets. The basal cells show similarly formed mitochondria distributed in the cytoplasm. With the bursting of the theca in stage II, some of the mitochondria may be lost, because in the following syncytial state (stage III), they are found in comparatively the same concentration as in stage I, but in a reduced amount of cytoplasm. No changes are apparent in the following stages, so that in the final stage (VI), the mitochondria are seen evenly distributed in the cytoplasm of the reformed cells (fig. 19). There is no apparent quantitative or qualitative change as compared to the tadpole stage (I).

The Golgi apparatus in the chief cell of the small intestine in the tadpole stage (I) consists of a heavy reticulum (fig. 20),

which completely surrounds the nucleus and extends as a more dense network into the cytoplasm above, and somewhat less densely, with only few reticular strands, below. In the network above the nuclei of some cells, small, non-staining droplets may be seen, and in others only one large drop is visible, probably having arisen through the coalescence of smaller drops. This large drop apparently migrates toward the lumen, and in so doing, may carry some of the Golgi substance with it. On reaching the apical border of the cell, the drops are seen to have greatly increased in diameter, filling the apical third of the cytoplasm as a typical goblet cell. In the passage toward the lumen most of the Golgi substance carried along disappears, but some few strands may occasionally still be seen in the cytoplasm close to the margins of the goblet. The secretory substance of the goblet apparently arises in close association with the Golgi apparatus.

In stage II, as already described, most of the chief cells pass through the goblet stage previous to rupturing at the free border. Simultaneously, and before the loss of cilia, the Golgi apparatus is seen to have undergone a change, passing into a fragmented state, similar to that found in the stomach. In this condition the Golgi substance is found as coarse granules spread throughout the cytoplasm with a greater concentration about the nuclei. With the bursting of the theca most of the granules are lost, so that in the syncytial condition (III), the Golgi substance can be seen as a granular ring about the nucleus, the cytoplasm being in general cleared of these granules, except for a dense accumulation bordering on the lumen. This dense accumulation is subsequently lost.

In the left limb stage (IV) the cytoplasm is for the most part cleared of the granules, and the Golgi apparatus is reformed as a thin, distinct reticulum which completely surrounds the nucleus. With the formation of the cell walls and elongation of the cells to their definitive columnar configuration, the reticulum becomes slightly more prominent. In the final stage (VI), the Golgi apparatus is seen as a thin, compact reticulum, completely surrounding the nucleus, with

perhaps some few strands extending into the cytoplasm above for a very short distance (fig. 22). There is distinctly less demonstrable Golgi substance than in stage I.

C. Pancreas

The acini of the pancreas in the tadpole stage (I) consists of a single layer of cells arranged about the secretory capillaries. A loose connective tissue is interspersed between the acini. In the hind limb stage (II) a general breakdown of tissue occurs. The acini are broken up and many of the cells are seen loosely scattered in groups throughout the tissue. Many of the cell groups appear necrotic, and an infiltration of phagocytes, chiefly eosinophiles, has occurred.

In stage III an increased amount of necrotic cellular material is seen. The greater part of this is probably phagocytized, but some is sloughed into a partially broken down pancreatic duct. Groups of retained cells, syncytial in nature, are scattered throughout the tissue. The secretory capillaries are invisible. In the following left limb stage (IV), most of the degenerated cellular material is no longer visible. There is a decrease in the number of phagocytes, and cell walls may again be discerned. We now find scattered groups of cells, with large spaces in the connective tissues between the groups. Numerous mitotic figures are seen.

In stage V a regenerated condition is apparent. The cell groups have arranged themselves into acini, and secretory capillaries are again visible. There is a noticeable compactness of tissue, due no doubt to the presence of newly formed cells together with the general contraction of the organ which in the final stage (VI) is much smaller than in the tadpole stage (I). The interstitial spaces are much reduced and there is a decrease of degenerated matter and phagocytes. This condition continues into the final stage (VI), except that the tissues are better differentiated and there is an absence of degenerated matter and phagocytes. This confirms the findings of Janes ('32) as described in his abstract.

The general cytological changes of the acinal cells are interesting. In the tadpole stage (I) the acinal cells are round or oval-shaped. The cytoplasm is densely granular, stains intensely with eosin, and contains some coarser, darkly staining granules. Prominent secretion droplets, staining dark blue with hematoxylin, are found concentrated in the apical regions of the cells, bordering the secretory capillaries (fig. 23). The The nuclei are usually located in the basal portion of the cell, and show coarse chromatic reticuli, making nucleoli indistinguishable.

In stage II we find a pronounced decrease in the number of droplets in the acinal cells. Some show a complete absence of droplets. With the general breakdown of the acini, the cell walls become indistinct. This loss of cell boundaries continues into the following stage until a complete syncytium of the retained cells obtains. In this stage (III), the nuclei are generally distributed in a finely granular cytoplasm, homogeneous in texture and containing a few droplets. The coarser granules as found in stage I are absent. Mitosis is rarely seen in this stage.

The left limb stage (IV) shows the beginning of cell wall formation. These cells show very little cytoplasm, the nuclei filling the greater part of the cells. The resting nuclei show coarse, darkly staining reticuli and nucleoli. Many of the cells are in various stages of mitosis. The cytoplasm is for the most part finely granular, but some few coarser, darkly staining granules may be seen. Small groups of darkly staining droplets are present. Where the cell walls are visible, the droplets can be seen concentrated near the capillary border.

In the right limb stage (V) cell walls are again present throughout. The condition as described above still persists, except that the cytoplasm takes a deeper eosin stain and is more densely granular. More of the coarser, darkly staining granules are visible, these being evenly distributed throughout the cytoplasm. In stage VI essentially the same condition obtains. The acinal cells in this stage seem, on the average, to

be somewhat smaller than in stage I. The cytoplasm is for the most part free of droplets (fig. 24), but where droplets are present, they are very few in number and are found concentrated at the apical border of the cell. The resting nucleus of the cell shows a faint reticular background and at least one or two distinct nucleoli.

No significant change, either quantitative or qualitative is to be noted in the mitochondria of the acinal cells during metamorphosis. In the tadpole stage (I) the mitochondrial bodies are seen as small, curved or rod-like forms, homogeneously scattered in the cytoplasm (fig. 25). Throughout metamorphosis the configurations and concentration of the bodies show no visible differences.

The Golgi apparatus in the tadpole stage (I) consists of long, heavy, isolated strands distributed in the clearer cytoplasm of the cell below the region where the droplets are concentrated (fig. 26). Clear droplets can be seen in close proximity to some of the strands; these droplets arising apparently either within or out of the Golgi substance. The free droplets in the neighborhood of the strands are seen to be surrounded by thin osmiophilic girdles which they apparently lose on migrating to the periphery.

In the hind limb stage (II) practically all the droplets are absent from the cytoplasm, and in those cells where the cell walls have disappeared, the Golgi substance is seen to be materially changed in character. The distinct heavy Golgi strands of the previous stage are now seen to be irregularly broken up, the fragments lying in a loose, chain-like manner (fig. 27). This condition is analogous to the fragmented state found in the other tissues. In the following stage (III), wherein the retained cells are syncytial, the fragmented Golgi substance is seen to be absent from the general cytoplasm, but is now found concentrated around the nuclei in the form of granular rings.

In the left limb stage (IV), with the beginning of cell wall formation, we find that the Golgi substance has apparently condensed, and is now for the most part seen as a thin

reticular layer, closely applied to the nucleus. This condition is essentially the same in stages V and VI, except that the reticulum is somewhat more prominent, and in the final stage (VI), some of the Golgi strands can be seen lying more freely in the cytoplasm (fig. 28). The Golgi configuration here differs markedly from that of stage I.

D. Liver

The liver differs from the other tissues studied in that very little degeneration of tissue occurs within it during metamorphosis, the greater part of the cells of the tadpole liver passing directly to the final stage without undergoing division or passing through a syncytial state.

The liver of the tadpole, stage I, consists essentially of compact groups of cells forming cords. Within the latter are the prominent bile canaliculi, and externally, the cords are surrounded by blood sinusoids. Flattened histiocytes line the sinusoids. With onset of metamorphosis, the cords are seen to be broken up (stage II). The bile canaliculi are for the most part obliterated, and the hepatic cells are more closely packed in groups. The sinusoids are somewhat wider, and the histiocytes are more prominent in that they are more rounded than in the previous stage.

In the opercular stage (III) some few small scattered groups of degenerated cells are visible. The sinusoids are much more prominent and many phagocytes, especially eosinophiles, are present. Practically no flattened histiocytes are to be seen. These have for the most part rounded up, and many have apparently become free in the form of lymphoid hemoblasts. Large numbers of these are found throughout the tissue. This condition is followed by a slight increase in the amount of degenerated material (stage IV). A greater number of phagocytes are present and phagocytosis is apparently occurring. Some of the degenerated material is, however, also lost by being sloughed into the bile duct, which becomes broken down in places. The lymphoid hemoblasts are now seen to be rapidly dividing.

In the right limb stage (V), the cells are again found to be arranged in definite cords, and the bile canaliculi again are visible. The lymphoid hemoblasts are still undergoing prolific division, but some, in the neighborhood of the reformed acini, are apparently flattening out and becoming histiocytes. Different stages of this flattening process can be seen. In the final stage (VI) the cords are for the most part established and lymphoid hemoblasts and phagocytes are very few in number.

The general cytological changes of the hepatic cells are fairly definite. The hepatic cells of the tadpole stage (I) are large polyhedral cells with large, generally round nuclei. The cytoplasm is finely granular except for the areas bordering the bile canaliculi; these are more densely granular and take a deeper stain (fig. 29). Non-staining droplets may be present in the basal portion of some cells. The nuclei show faint reticular backgrounds and one or two distinct nucleoli. In the following stage (II) the denser areas bordering on the bile canaliculi are seen to have disappeared. The cytoplasm becomes generally vacuolated, and coarse, dark granules and droplets are scattered throughout. The cells appear smaller than previously.

In the opercular stage (III) the droplets and most of the coarser granules are absent, the granules persisting longest at the basal border. This condition is essentially the same in the following stage. In stage V we find the cytoplasm finely granular with a few droplets again making their appearance in some cells. These are found scattered in the basal portion of the cell. Those droplets nearest the cell wall take a dark hematoxylin stain. This condition persists into stage VI (fig. 30). The only visible difference between the cells here and in stage I, as observed in hematoxylin-eosin preparations, is the absence of the denser, more granular areas bordering on the bile canaliculi of the liver.

The mitochondria in hepatic cells of the tadpole are long slender threads. These are seen to be more or less oriented so that their long axes are parallel to the planes running from

the bile canaliculus to the blood sinusoid bounding the cell (fig. 31). In stage II through to stage V the threads are no longer oriented (fig. 32), but are found irregularly scattered in the cytoplasm sometimes partly clumped together. In the final stage (VI), with the reformation of the canaliculi and sinusoids, the mitochondria are again found to be oriented in a similar manner as in stage I, and the mitochondrial configuration is apparently the same.

The Golgi apparatus in stage I is seen to consist of a heavy reticular structure, polarized at that border (or borders) of the cell adjoining the bile canaliculus. It is concentrated only immediately around the canaliculus, as is seen in cross section, and may therefore be characterized as a reticular band running parallel to the canaliculus. Some strands of the reticulum may extend a short distance into the cytoplasm (fig. 33). In stage II, we have the appearance of a fragmented condition as in the other tissues (fig. 34). Here the Golgi substance is seen to be granular in nature and confined to the zone originally held by the reticulum. This fragmented condition persists through to the next stage, but in stage IV, its reticular nature is reestablished in part. The Golgi substance is now seen as a very thin, compact reticular strip lying close to the cell wall, adjacent and parallel to the bile canaliculus. In stage V, the reticulum is slightly more prominent, and in the final stage (fig. 35), it is seen to consist of a fine network, somewhat less compact than in the previous stage, but much reduced as compared to stage I.

DISCUSSION

No reference was found in the literature which correlates the histological changes which occur in normal metamorphosis with those which occur during precocious metamorphosis. The most extensive histological investigations have been carried out on the alimentary tract, and in all cases the authors have confined themselves to studying the changes which occur during either normal metamorphosis (Ratner, 1891; Reuter, '00 b; Deusberg, '06; Bowers, '09; Kuntz, '24),

or thyroid accelerated metamorphosis (Lim, '19; Champy, '22; Janes, '34). The general gross histological changes in the stomach and intestine of both normally and precociously metamorphosed forms seem apparently to be similar. In both cases there is noted loss of cilia in the epithelium of stomach and intestine, degeneration and subsequent regeneration of portions of the epithelium and glands of the stomach and epithelium of the intestine, increase in thickness of submucosa and muscle layers, and general contraction of both the stomach and the intestine. The gross histological changes occurring during the thyroid accelerated metamorphosis in R. catesbiana, observed during the course of the present work, are in agreement with those already reported. However, even though the histological changes as reported for normal and precocious metamorphosis are grossly similar, there may nevertheless be differences existing in the degree of changes and the relative time required for such changes. Jordan and Speidel ('23) indicate that the stress under which the organism is placed during thyroid feeding finally results in metamorphic stasis. The normally metamorphosing form does not have to contend with such a stress. Champy ('22) reports that the administration of thyroid to frog larvae calls forth two stimulative effects, one degenerative, the other regenerative. He indicates that with stronger feeding, especially with preparations richer in iodine, the tissue degenerative element is stronger and vice versa. Gudernatsch ('30) indicates that animals metamorphosed with thyroid lose much more water than do the controls. These reports seem to indicate the need for further details of comparative study and the establishing of some quantitative methods of comparison.

The question of how the definitive epithelium of the stomach and intestine is regenerated has occupied the attention of several investigators. Reuter ('00 b), states that in Alytes obstetricans the basal cells in the mucosa of the intestine are special absorbing cells, and that they are all sloughed off during metamorphosis. Deusberg ('06), for R.

fusca, Bowers ('09) for B. lentiginosus and Janes ('34), for R. clamitans, report that the basal cells undergo mitosis during metamorphosis, thus giving rise to the definitive epithelium. In additon, Janes ('34) notes that some of the larval columnar epithelial cells are retained and undergo division to give rise to definitive epithelium. In the present work the basal cells in stomach and intestine are found undergoing mitotic division and many of the larval columnar cells are retained. It is interesting to note in this regard that adequate proof is lacking concerning the division of these retained columnar cells. As indicated, practically all the mitotic figures in the epithelia of the stomach and intestine, were found in the deeper regions of the regenerating epithelia. It is probable, therefore, that only the basal cells undergo division and most of the retained columnar cells pass through to the final stage without dividing.

The present work is in agreement with the observation of Nassanov ('23), in Triton and Axolotl, that the goblet cells in the intestinal epithelium arise from the chief cells. Nassanov, however, indicates that the goblet cells may also arise from the compensatory (basal) cells lying deep in the intestinal folds. The present finding in R. catesbiana is at variance with this in that the goblet cells were not found to rise directly from the basal cells, but from the chief cells.

The factors involved in the removal of degenerated matter from the alimentary tract has been analyzed by Kuntz ('24) in R. pipiens and A. tigrinum, and Janes ('34) in R. clamitans. Both describe the phagocytes as playing an important role in the removal of necrotic material. Bowers ('09), in B. lentiginosus, could however find no evidence that phagocytosis occurs in the epithelium of the intestine. Jordan and Speidel ('23), in R. catesbiana state that the eosinophiles are not phagocytic in the intestine. The present findings are in agreement with Jordan and Speidel ('23) for both the intestine and stomach. Jordan and Speidel believe that some lytic substance is liberated by degenerating eosinophiles, which causes the degeneration of tissue. It is, however, significant that considerable necrotic matter is already present in the stomach and

intestine early in metamorphosis (stage II) before any appreciable number of phagocytes are present. The liberation of a lytic substance is yet to be demonstrated. Sloughing seems to be the chief method by which the stomach and intestine rids itself of degenerated matter. In the pancreas and liver phagocytosis seems to be the major factor in the removal of necrotic matter, while the sloughing process occurs to a minimum, being limited to the pancreatic and bile ducts.

The observed breakdown of the acini in the pancreas, confirms Lim's findings in R. temporaria ('19), and more recently Janes' ('32). Lim indicates that the acini break up into individual cells. The present study indicates that the retained cells tend to remain in groups which are syncytial in nature during the early stages of metamorphosis and then undergo a series of changes terminating in the definitive cells of the pancreas.

Reduction in the amount of mitochondria has been observed in several types of cells. Guilliermond ('12) reports that in general the amount of mitochondria is reduced in plant cells as they grow older, and Cowdry ('16), describes a definite reduction in the cells of the skin as they migrate up to the surface. Cowdry also reports a decrease in the amount of mitochondria during the process of enucleation in erythropoiesis. In general, these reported decreases are correlated with cellular maturation. In the present work an apparent decrease in the number of mitochondria in the gastric gland cells is observed during metamorphosis. This decrease first becomes evident in stage II, and persists through to the final stage (VI). The other cells studied show no apparent difference in this respect. This decrease in the number of mitochondria cannot be interpreted at this time. It seems unlikely that cellular maturation is a factor here.

The origin of secretory droplets from, or in close proximity to, the Golgi apparatus in the intestinal epithelium and in the cells of the pancreas, as described, is not new to the literature of cytogenesis, and it may be considered in the light of Bowen's statement ('29), that "Apparently the Golgi ap-

paratus plays some immediate role in the process of accumulation and final synthesis of the secretion product. . . ." Nassanov ('23), in the intestine and pancreas of Triton and Axolotl, and Saguchi ('20), in the pancreas of R. temporaria also report the origin of secretory droplets in proximity to the Golgi apparatus.

It is a more or less accepted opinion that the Golgi configuration in cells varies with the physiological conditions obtaining (Cowdry, '24; Bowen, '29; Nahm, '33; Sharp, '34). Cramer and Ludford ('25), show, for example, that the Golgi reticulum in the rat's intestine becomes much denser and heavier during fat absorption. In R. catesbiana, as reported, a reduction in the amount of demonstrable Golgi substance occurs during metamorphosis, so that in the final stage (VI), a much reduced, and less dense reticulum, as compared to stage I is observed. The cause of this reduction is difficult to ascertain, but in view of previous evidence, it is safe to assume that physiological factors are involved. Thyroid treated animals ingest little or no food during metamorphosis and are also subject to high metabolic rates. This undoubtedly leads to a depletion of body stored foods. Since the Golgi substance is generally believed to consist of lipoid compounds (Tennent, Gardiner and Smith, '31; Nahm, '33), or lipoids and proteins (Bowen, '26), the reduction here observed may be explainable in that either some of the Golgi substance is metabolically used up or that the Golgi substance is reduced because it is inactive, or only slightly active due to the depletion of certain compounds which are necessary for its activity. Perhaps all these factors are involved.

The fragmentation of the Golgi apparatus which was observed in all cells studied is interesting. In only a few other cases is the fragmentation of Golgi substance known to occur. Bowen ('26), states that the Golgi apparatus universally breaks up during the growth period of eggs, and that the Golgi substance in the mature eggs is always in the form of scattered bodies (with the possible exception in mammals). Cajal ('14) reports that when ordinary connective tissue cells are converted to adipose tissue, the Golgi network undergoes a

process of fragmentation, and Bowen ('26) reports similar fragmentation in lipoid glands of many types. Nothing is known as to the cause of this fragmentation. In view of the probable chemical nature of the Golgi substance, certain chemical changes or physical changes, such as surface tension changes may, in all probability, be the causative factors. It is interesting to note that fragmentation, as observed here, is always accompanied by radical cytological changes such as the loss of secretory granules or droplets. The interrelationship of the two facts is, however, unknown. The behavior of the Golgi substance may afford an excellent criterion for following the course of precocious metamorphosis. It offers the best, and as far as studied, the most universal means for following metamorphosis microscopically. It would be interesting to determine whether the behavior is similar in normal metamorphosis.

Lim ('19) in R. temporaria reports that the numerous mitoses seen in the liver during metamorphosis are almost wholly limited to the red blood corpuscles. In R. catesbiana, the many mitotic divisions seen in the liver during metamorphosis are practically restricted to the lymphoid hemoblasts (terminology of Dawson, '33) which apparently arise for the most part from the histiocytes found close to the boundaries of the sinusoids. Jordan and Speidel ('23) find that the new metabolic rate obtaining in the tadpole of R. catesbiana during metamorphosis calls forth a demand for lymphoid hemoblasts as well as other cellular blood elements. The lymphoid hemoblasts are progenitors of erythrocytes, granulocytes, phagocytes and, in addition, serve as promoters of cell growth in the regions of progressive change. Jordan and Speidel ('23) show that the hemopoietic centers, especially the spleen and kidneys, become depleted of their hemopoietic capacities during metamorphosis. In this study it was observed that the hemopoietic capacity of the liver is most strongly called upon in stages III and IV; quite advanced in metamorphosis. It seems, therefore, that the liver is one of the later centers drawn upon for hemopoietic purposes.

SUMMARY

1. Gross histological changes in stomach, intestine and pancreas during the thyroid accelerated metamorphosis of R. catesbiana larvae are in general agreement with previous observations.

2. In the pancreas much of the tissue degenerates, but retained cells regenerate to form the definitive tissue.

3. Most of the cells in the liver carry through to the final stage without undergoing division. Very little degeneration occurs.

4. The hemopoietic capacity of the liver is drawn upon during metamorphosis, involving the transformation of histiocytes to lymphoid hemoblasts.

5. Cytological changes were observed in the epithelial and gland cells of the stomach, the epithelial cells of the intestine, the acinal cells of the pancreas, and hepatic cells of the liver. A loss of secretory granules and droplets occur in the various cells studied during metamorphosis.

6. An apparent quantitative decrease in mitochondria occurs in the gastric gland cells.

7. The Golgi apparatus in the cells of the epithelium and glands of the stomach, epithelium of the intestine, acini of the pancreas and cords of the liver undergo analogous changes at approximately the same metamorphic stages. The Golgi substance fragments in the early stages of metamorphosis to recondense later. In the final stage the demonstrable Golgi appears much reduced.

8. Changes in the Golgi configuration, mitochondria, secretory granules and droplets are correlated with external changes. The constant modifications of the Golgi architecture is proposed as a criterion for microscopically following the course of metamorphosis in thyroid treated amphibian larvae.

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PLATE 1

EXPLANATION OF FIGURES

Figures drawn with camera lucida. Epithelial cells of stomach and intestine drawn × 900. All other cells drawn × 1200.

1 Stomach epithelial cell. Stage VI, showing mitochondria. Champy-Kull.

2 Stomach epithelial cell. Stage I, showing same distribution of mitochondria. Champy-Kull.

3 Stomach epithelial cell. Stage I, ciliated. Bouin, hematoxylin-eosin.

4 Stomach epithelial cell. Stage VI, non-ciliated. Zenker, hematoxylin-eosin.

5 Stomach epithelial cell. Stage I, showing Golgi. Nassanov-Koletschov.

6 Stomach epithelial cell. Stage II, showing fragmentation of Golgi. Nassanov-Koletschov.

7 Stomach epithelial in syncytial stage, stage IV, showing Golgi. Nassanov-Koletschov.

8 Stomach epithelial cell. Stage VI, Golgi. Nassanov-Koletschov.

9 Stomach gland cells. Stage I, showing coarse granulation. Zenker, hematoxylin-eosin.

10 Stomach gland cells. Stage VI, showing absence of coarse granules. Zenker, hematoxylin-eosin.

11 Stomach gland cells. Stage I, numerous mitochondria. Champy-Kull.

12 Stomach gland cells. Stage VI, showing less mitochondria. Champy-Kull.

13 Stomach gland cells. Stage I, Golgi. Nassanov-Koletschov.

14 Stomach gland cells. Stage II, fragmentation of Golgi. Nassanov-Koletschov.

15 Stomach gland cells in syncytium. Stage IV, Golgi compact. Nassanov-Koletschov.

16 Stomach gland cells. Stage VI, Golgi. Nassanov-Koletschov.

17 Intestinal epithelial cells. Stage I, ciliated. Bouin, hematoxylin-eosin.

18 Intestinal epithelial cell. Stage I, mitochondria, Champy-Kull.

19 Intestinal epithelial cell. Stage VI, non-ciliated, showing mitochondria. Champy-Kull.

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PLATE 2

EXPLANATION OF FIGURES

20 Intestinal epithelial cells. Stage I, Golgi. Nassanov-Koletschov.

21 Intestinal epithelial cells in syncytium. Stage III, showing Golgi fragmented and granules being cast into the lumen. Nassanov-Koletschov.

22 Intestinal epithelial cell. Stage VI, Golgi reduced. Nassanov-Koletschov.

23 Pancreatic acini cells. Stage I, showing secretion droplets. Bouin, hematoxylin-eosin.

24 Pancreatic acini cells. Stage VI, no droplets. Zenker, hematoxylin-eosin.

25 Pancreatic acini cells. Stage I, mitochondria. Champy-Kull.

26 Pancreatic acinus cell. Stage I, showing Golgi and osmiophilic girdles around some droplets. Nassanov-Koletschov.

27 Pancreatic acinus cell. Stage II, fragmentation of Golgi. Nassanov-Koletschov.

28 Pancreatic acinus cell. Stage VI, Golgi. Nassanov-Koletschov.

29 Liver cells. Stage I, showing dense granular areas at bile canalicula. Bouin, hematoxylin-eosin.

30 Liver cells. Stage VI, showing absence of dense areas. Zenker, hematoxylin-eosin.

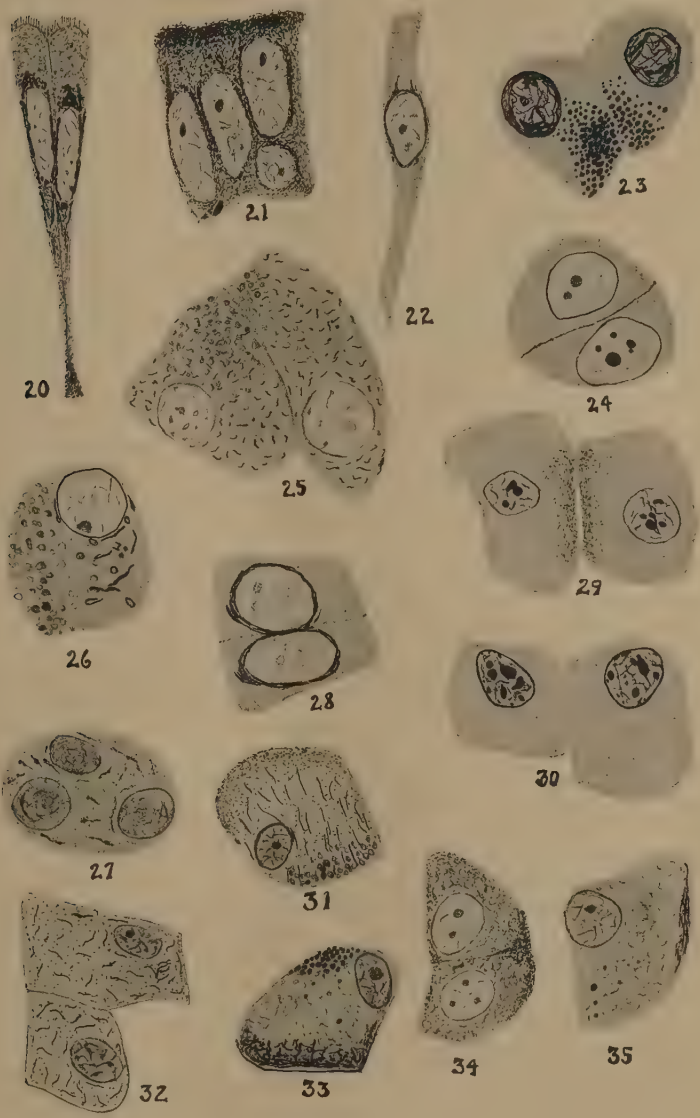
31 Liver cell. Stage I, showing mitochondria oriented. Champy-Kull.

32 Liver cells. Stage II, mitochondria not oriented. Champy-Kull.

33 Liver cell. Stage I, Golgi. Nassanov-Koletschov.

34 Liver cells. Stage II, Golgi fragmented. Nassanov-Koletschov.

35 Liver cell. Stage VI, Golgi again reticular. Nassanov-Koletschov.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STUDIES ON THE GROWTH OF THE HUMAN NERVOUS SYSTEM

IV. MATERIAL ILLUSTRATING THE POSTNATAL GROWTH AND TOPOGRAPHY OF THE BASAL NUCLEI

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TWO TEXT FIGURES AND ONE PLATE

Relatively little is published on the postnatal growth and topography of the basal nuclei of the human cerebrum, although Franceschi (quoted by Donaldson, 1895) has recorded some figures regarding their size in children and adults.[1]

H. L. Dunn, in a study of the growth of the brain, sectioned and photographed twenty-five specimens of various ages. He has kindly allowed us to use twelve specimens of this series for a further illustrative study by reconstruction and graphic methods; the results are presented here.

METHODS

The material was prepared in the following way:

All the specimens were fixed in a 10 per cent formalin solution for at least 6 months. Each brain was then placed in a mass of melon pulp and concentrated aqueous gelatin solution and the whole allowed to set over night. The matrix was

[1] Franceschi (1888) weighed the corpora striata and the optic thalami. His method of preparing this subdivision was to excise the basal ganglia with the cortex of the insula attached, and then to dissect away both the cortex and the white matter of the insula until the gray substance of the ganglia could be clearly seen. The weights of these masses given by Franceschi are about twice those of the nuclei obtained in this study. This may be explained by his inclusion of the white substance of the corpora striata. The relative increase in these masses as noted by Franceschi is about the same as that obtained in this study of the basal nuclei proper.

frozen in a brine solution, but the brain was not allowed to freeze, for this process would distort it. The mass with the embedded brain was then fastened securely in a meat-slicing machine and cut in sections approximately 3 mm. in thickness. Tracings in India ink were then made directly from these sections on sheets of commercial kodaloid.

Wax plates were cast of the same thickness as the original sections and outlines of the tracings transferred to them. The areas representing the cortex and the basal nuclei were cut away and those portions representing the white substance discarded. Wax reconstructions were then made in the usual manner and a small jet of flame applied to them sufficient to make the plates adhere. Windows were cut in the lateral walls of the models to show the basal nuclei. Separate models were also made of the basal nuclei of the cerebrum. These were slightly smoothed by the flame method, without addition or loss of wax.

SUMMARY OF FINDINGS

Growth of the basal nuclei

An approximate estimate of the growth in volume of the cerebrum and of the basal nuclei may be made by volumetric measurements of these models. This is illustrated by the values given in table 1.

The reconstructions indicate that the cerebrum increases approximately two and one-half fold between birth and maturity. By 2 years the cerebrum has attained one-half of its mature size. The basal nuclei increase nearly threefold in postnatal life. Their greatest growth is in infancy. The rapid growth of these structures is also shown in figures 1, A, B and C, which are drawings of reconstructed nuclei.

No published measurements of the total length of the whole basal nuclear mass were found, but there are several measurements of the component parts. Poirier and Charpy ('01) gave the length of the caudate nucleus as ranging from 60 to 70 mm.; the lenticular nucleus, 45 to 50 mm. in length. Dejeriné's (1895) measurements were 65 to 70 mm. for the length of the

caudate nucleus and 35 to 50 mm. for the length of the optic thalamus.

Mensuration of the component parts of the basal nuclear mass was not attempted in this study, but measurements were made of the reconstructions as a whole. The length of the nuclear mass in the models varied from 40 mm. in the newborn to 66 mm. in an adult. The reconstructed nuclei of adult brains alone varied in length from 51 to 66 mm.

TABLE 1

Cubic displacement of reconstructions of a series of human cerebri and their basal nuclear masses

NUMBER	AGE	VOLUME OF RECONSTRUCTED CEREBRUM	VOLUME OF RECONSTRUCTED BASAL NUCLEI
	years	*cc.*	*cc.*
1006	Newborn	395	14.5
1017	0.17	322	..
1004	0.22	400	20.0
1002	0.315	505	26.0
1018	0.44	455	30.5
1000	1.23	314	17.0
299	2.00	545	21.0
1011	23	..	37.0
1012	26	1210	53.0
1007	44	1010	47.0
1009	49	1000	49.5
1014	Adult (age unknown)	935	40.5

Numbers 1000 (age 1.23 years) and 299 (age, 2.0 years) were each only one-half brains. The nuclei of 1017 (age, 0.16 years) were not reconstructed for the specimen was badly crushed in embedding.

Topography of the basal nuclei

Although the topography of the ventricular system of the cerebrum is quite well known, particularly since the introduction of ventrilography, the position of the basal nuclear masses with regard to the configuration of the lateral surface of the cerebrum has received little attention. Apparently this topography changes comparatively little in postnatal life. In every instance the anterior pole extends into the inferior frontal gyrus.

There is much variation in the angle at which the lateral fissure crosses the nuclei. In the newborn brain the posterior ramus of the fissure is inferior to the anterior half of the nuclear mass. It then curves upward and crosses the mass at

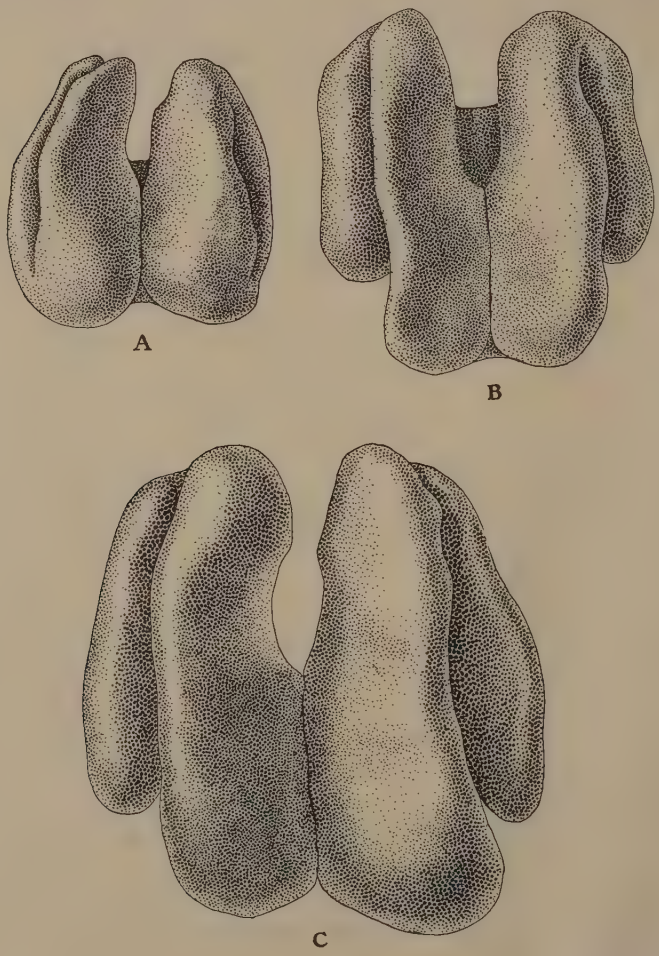

Fig. 1 Superior surfaces of reconstructions of basal nuclei of the human cerebrum. Three-fourths actual size. A, newborn infant (no. 1006). B, infant of 0.22 years (no. 1004). C, adult of 26 years (no. 1012).

an angle of approximately 45°. The anterior ascending ramus of the fissure corresponds, approximately, to the anterior boundary of the nuclei.

In the brain of an infant of 0.22 years a very small portion of the posterior pole of the nuclear mass lies in the superior temporal gyrus. The anterior ascending ramus of the lateral fissure passes upward over the extreme anterior part of the basal nuclear mass at an angle of approximately 90°.

The adult brains show some difference in the position of the nuclei with regard to the fissure, although there is considerable variation in this relationship. In a model of the brain of an adult of 23 years the lateral fissure passes over the anterior half of the nuclei and crosses them at an angle of approximately 45°. In the other models of the adult brains the posterior ramus of the lateral fissure lies over the lower third of the nuclear mass. In these models, as in those of infant brains, the anterior end of the posterior ramus of the lateral fissure marks the anterior boundary of the nuclear masses.

In maturity the posterior pole of the nuclear mass commonly extends well below the lateral fissure, often to the level of the superior temporal sulcus, a relationship that we have not observed in younger brains. Thus the nuclear mass is placed lower in the adult than the child's brain. This is probably due to the growth of the inferior part of the nuclear mass, but we cannot exclude the possibility of some shifting of the position of the fissures during postnatal development.

Figures 2, A, B and C are plates of translucent models of the cerebrum showing the enclosed nuclear masses in the adult, and figures A, B and C of plate 1 are similar illustrations of the cerebrum and nuclei during the growth period. The basal nuclei are seen as two masses, the lateral being the caudate nucleus and the medial, the lenticular nucleus, together with the optic thalamus.

There seems to have been no systematic study of the topography of the basal nuclei with respect to the cerebral surface, although the relationship has been illustrated in a few

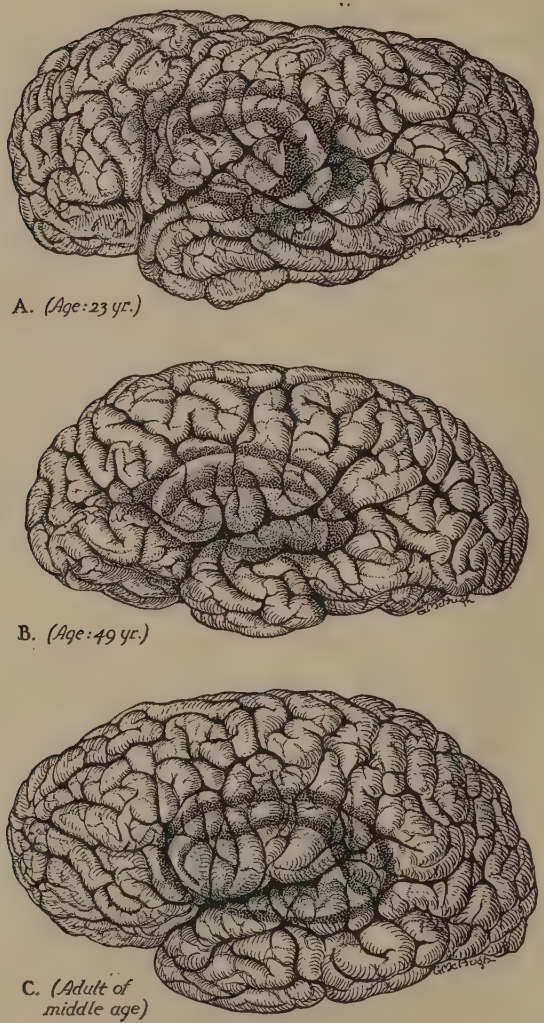

Fig. 2 Lateral views of models illustrating the topography of the basal nuclei in the adult human cerebrum, drawn as translucent objects, showing the position of the basal nuclei. One-half actual size. A, adult of 23 years (no. 1011). B, adult of 49 years (no. 1009). C, adult of unknown age (no. 1014).

schematic drawings. The present findings are not in complete agreement with these schemata.

In the atlas of Sobotta-McMurrich (edition of '09, p. 179, fig. 672) the nuclei are represented as placed almost entirely in front of the anterior bifurcation of the lateral fissure, and the same diagram shows the lower pole of the thalamus projecting into the middle temporal gyrus. This figure is eliminated from the recent English edition of this work ('30).

A corresponding diagram by Merkel (1891, vol. 1, p. 129, fig. 75) shows the lenticular nucleus lying in front of the anterior end of the lateral fissure, and the anterior pole of the nuclear mass inferior to the lateral fissure.

In a diagram by Wilson (1894) the nuclear mass is shown projecting in front of the anterior end of the posterior ramus of the lateral fissure, which approximately bisects it.

Poirier and Charpy ('01, vol. 3, p. 358, fig. 253) show the lenticular nucleus placed in front of the anterior end of the lateral fissure. The portion lying inferior to the horizontal portion of the fissure is not definitely indicated.

A reconstruction by Symington ('03) of the head of a female of 54 years shows a stippled area in the region occupied by the nuclei but the labeling is so obscure that we could not identify it with certainty.

RÉSUMÉ

Reconstructions indicate that the cerebrum as a whole increases about two and one-half times in volume in postnatal life. By two years it has attained one-half its adult size. The basal nuclei increase nearly threefold: their greatest growth is in infancy.

The reconstructions of the basal nuclei at natural size vary in length from 40 mm. at birth to a maximum of 66 mm. in maturity. The reconstructions of the nuclei of adult brains vary in length from 51 to 66 mm.

The position of the nuclear mass with respect to the lateral surface of the cerebrum shows comparatively little antero-posterior change after birth. The anterior pole extends well

into the zone of the inferior frontal gyrus. The inferior border of the mass lies more inferiorly in the temporal lobe in the adult than the infant.

There is considerable variation in the angle at which the posterior ramus of the lateral fissure crosses the nuclei. In the newborn the fissure is inferior to the anterior half of the nuclear mass. In adult brains the posterior ramus usually lies over the lower third of the nuclei.

The anterior ascending ramus of the lateral fissure marks roughly the anterior boundary of the nuclear mass in both infant and adult.

MATERIAL

Twelve brains were used in this study. The history of each, insofar as obtainable, is as follows:

1) No. 1006. Full-term newborn. No significant history. Brain in good condition. 2) No. 1017. Age, 0.17 years. Death by inanition. Brain in fair condition, but somewhat distorted in embedding. 3) No. 1004. Age, 0.22 years. No significant history. Brain in good condition. 4) No. 1002. Age, 0.315 years. No significant history. Brain in good condition. 5) No. 1018. Age, 0.44 years. Death from bronchopneumonia. All organs normal, all lymph nodes large and prominent. Brain in good condition. 6) No. 1000. Age, 1.23 years. No significant history. Brain in good condition, but only one complete lateral half available. 7) No. 299. Age, 2.0 years. Death from purpura hemorrhagica. Autopsy record shows hemorrhages into lungs, endocardium and subcutaneous tissues and marked cervical adenopathy. One complete lateral half of the brain available. 8) No. 1011. Age, 23 years. Basilar leptomeningitis (streptococcic), cloudy swelling liver and kidneys, septic spleen. Brain in very good condition. 9) No. 1012. Age, 26 years. Nephrosis. Pia and arachnoid thickened and slightly opaque. No gross lesions in brain. Brain in excellent condition. 10) No. 1007. Age, 44 years. Luetic cirrhosis of the liver, cirrhotic spleen, pleurisy and bronchitis, ascites, tuberculous lymphadenitis, chronic polyserositis Brain in good condition. 11) No. 1009. Age, 49 years. Death from lye poisoning (suicide). All organs negative. Brain showed some irregularity and atrophy, but otherwise in good condition. 12) No. 1014. Adult, exact age unknown. No significant history. Brain excellently preserved.

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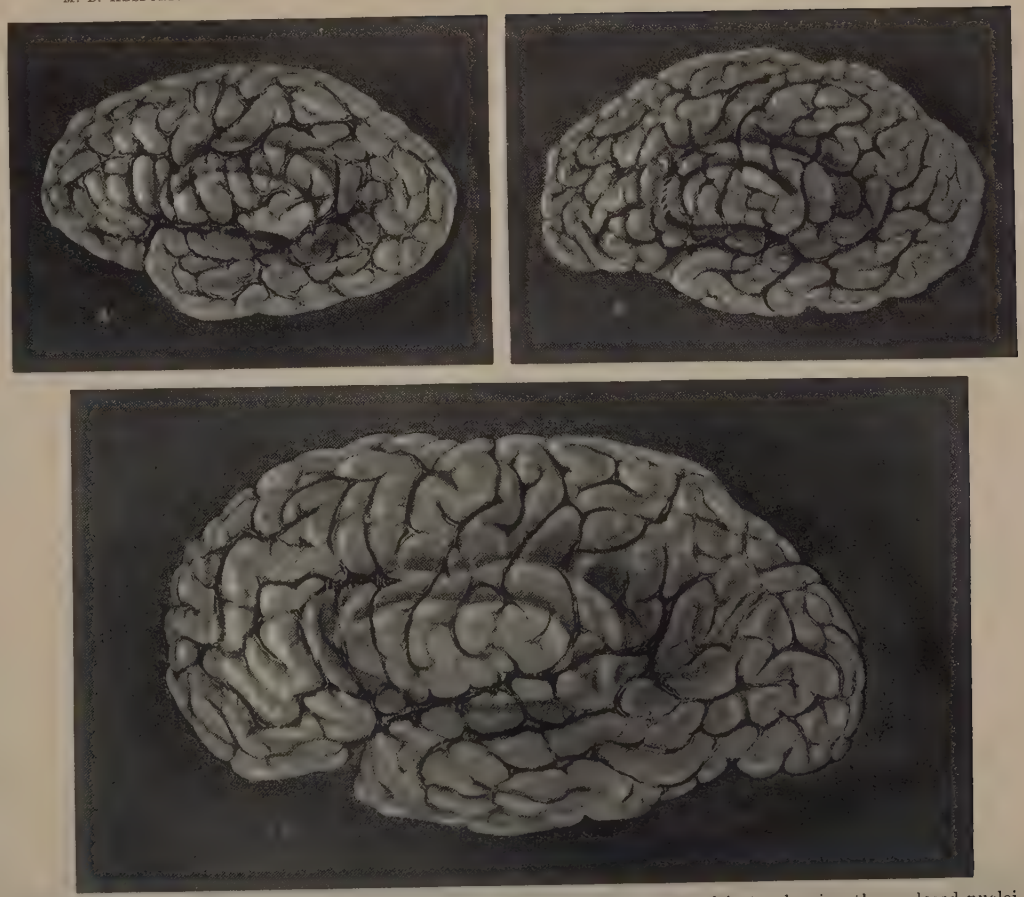

Lateral views of models of cerebri of various ages, drawn as translucent objects, showing the enclosed nuclei. One-half actual size. A, newborn infant (no. 1006). B, infant of 0.22 years (no. 1004). C, adult of 26

SEASONAL SEXUAL ACTIVITY AND ITS EXPERIMENTAL MODIFICATION IN THE MALE SPARROW, PASSER DOMESTICUS LINNAEUS [1]

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THREE TEXT FIGURES AND TWO PLATES (SIX FIGURES)

The seasonal character of reproductive periodicity in the wild bird is substantial evidence for the belief that some environmental factor is concerned in the production of the sexual cycle. According to Rowan ('25 to '29), Bissonette ('30 to '33), and Cole ('33), the incidence of daily light is an extremely important factor.

The present investigation was undertaken with the hope of extending our knowledge relative to sexual activity in the bird under field, laboratory, and experimental conditions. The English sparrow proved to be very favorable material for critical study.

LITERATURE REVIEW

Schafer ('07) suggested that the constant change in the number of hours of daily light might logically be considered a factor in the avian sexual cycle, inasmuch as the cycle is so regular, and the variation in the number of hours of daylight is one of the most consistent and regular of natural changes. It was Schafer's claim that light period is the causative factor in the migration of birds. According to him, birds migrate to regions where there is more daily light so that they may

[1] The studies presented in this investigation were carried out in the Department of Zoölogy.

have longer time during which to feed. In the southern habitat, with increased light and food consumption, the gonads enlarge.

Within recent years the results of a number of studies on the experimental modification of sexual activity have appeared in the literature as an indirect result of the contentions of Schafer. Rowan determined to ascertain experimentally whether the incidence of daily light is actually a factor in seasonal sex activity and migration. In 1925 he reported his experimental results on the reactions of juncos to added daily illumination. The birds were trapped and caged while passing through Edmonton, Alberta, on their southward migration. On October 2nd the caged birds were exposed to an electric light for a period of 5 minutes following sunset. Each succeeding day the exposure to light was lengthened by 5 minutes. Rowan observed that the testes of experimental juncos were in the spring state by December 30th, whereas caged controls which did not receive light treatment retained small testes. The experiments were carried out in the bitter cold of Alberta (—44°F.). In the mild climate of California, at the same time of the year, juncos in the natural habitat showed small testes, indicating that temperature is not a factor of great consequence in causing periodic sexual activity. Rowan ('28) concluded from further experimental studies that added activity, afforded by the increased daily light, was the cause for increase in testis size. Artificial activation of juncos after dark (without any bright illumination) from March 17th to April 28th caused an advance in testis size comparable to that resulting from light treatment.

Bissonnette ('30, '31), using the European starling as experimental material, found, contrary to Rowan, that light rather than activity was the controlling factor in the increased spermatogenic activity of the birds. Cutting down the daylight period from March 19th to April 15th produced regression of the testis. Although regression was less marked in artificially activated birds, and in some birds it did not occur, Bissonnette concluded that activity can at most but modify the reaction to

light. Fundamental proof for Bissonnette's claim, namely, that light is the factor primarily responsible for the sexual cycle, is furnished by the subsequent observations.

Bissonnette ('32) reported that red light stimulates testis development to a greater degree than white light; green light inhibits development of the testis. Inasmuch as different types of light produce different reactions, Bissonnette believes that light is the primary factor involved. Increasing the intensity of light hastens development to a certain degree, but beyond a certain light intensity marked effects are not evident.

Bissonnette ('32) demonstrated that diet may modify the reactions of starlings to light. Birds kept on a rich diet may be brought to full spermatogenic activity within 20 days by red light. On a restricted grain mash diet, however, the very stimulating red light fails to induce testis activity even in 22 days. Food may be a limiting factor in the appearance of sexual activity, although the daily light ration is adequate. Bissonnette believes that there is a deep-rooted physiological basis for the sexual cycle. Once the peak of spermatogenesis is reached regression follows, even though the birds receive additional light. Regression of the starling testis in nature starts before June 21st, when the maximum number of hours of daily light is reached.

MATERIALS AND METHODS

The sparrows were collected at 1- to 2-week intervals from October to the middle of April. They were either trapped and brought alive to the laboratory or shot directly in the field. The live birds were kept in a laboratory animal room where they were protected from the wind and extreme cold. The cages were of frame construction, with the top and front covered with a fine meshed screen. Adequate provision was made for roosting. Millet, hemp, and wheat seeds were fed the birds, food and fresh water being available at all times.

Birds placed in the experimental 'light cage' were given added light after sunset from an ordinary 60-watt incandescent bulb which was placed slightly over a foot away from

the roost of the cage. Lighting was started October 21st. By half-hour daily increases the birds were receiving 6 hours of added daily light on November 1st. From this time to November 9th inclusive the added daily light ration was kept constant at 6 hours. On November 10th the ration was increased to 6½ hours because the days were growing shorter and the lights were being turned on earlier. On November 28th the added light period was increased to 7 hours. All 'light birds' from then on received 7 hours of additional daily light until killed for examination. The control birds were receiving only ordinary daylight during this period.

In a recent check experiment the conditions, however, were somewhat different. A 100-watt incandescent lamp was hung about 3 feet above the roost of the birds. The experiment was carried out later in the season, from early December to January. Lights were kept on for 6 hours after dark from the first experimental day until the time the birds were killed.

The daily light ration was decreased by placing a black cover over the experimental cage. The cover was mounted on casters to facilitate manipulation. Birds in the 'decreased light cage' received 5 hours of ordinary daylight from January 17th to February 17th. From the latter date to March 21st the birds received only 4 hours of light daily. Subsequent to March 21st the daily light was decreased to 3 hours, the source of light being ordinary daylight which passed through the windows of the room in which the birds were kept. The control birds were in an uncovered cage. The amount of light which they received, however, was less than that birds in nature received. In both the increased and decreased light experiments the birds were receiving little or no ultra-violet light.

During the months that the experiment in increased daily light was conducted the temperature in the room varied from 12°C. to 23°C. After 7 hours of lighting the temperature in the 'light cage' was sometimes 3° higher than in the control cage. The great temperature variation, however, during the course of the experiment made this difference an inconsequential factor.

Most of the gonadal material was fixed in Bouin, although Helly fixation was used on several occasions. Sections were cut at 5, 6, and 10 μ. Heidenhain's iron hematoxylin was the stain commonly employed. In cases of counterstaining acid fuchsin orange G was used.

In view of the fact that gonadal material for the study of normal sexual activity was fixed in the field, testis measurements were made subsequent to fixation. Measurements of the control and experimental material were also made following fixation. Weights of the testes, when given, were based on material previous to fixation.

The measurements of extremely small gonads were made under a dissecting binocular provided with a calibrated eyepiece. Large testes were measured by placing the dish containing the gonads (in 70 per cent alcohol) over a ruler graduated in millimeters. The measurements of small gonads are accurate to two decimal places, those of larger gonads to one.

Testis volume was calculated by means of the formula $V = \frac{4}{3}\pi ab^2$ (for computing volume of ellipse). 'a' represents the major axis and 'b' the minor axis of the testis (Bissonnette, '30).

OBSERVATIONS

Normal sexual cycle

Gross changes in the gonads.[2] These observations are based wholly on the sexual cycle of birds found free in nature. Critical analysis of the sexual cycle is of real value only when based on determined material, that is, adult or juvenal. Differentiation between the adult and juvenal sparrows is possible in the fall and early winter on the basis of a number of characters. The beaks of adult males are black in color,

[2] According to Newton and Gadow (1894), the sparrow testis ranges in size from that of a mustard seed to a small cherry, depending on the season. Leuckart (cited by Loisel, '00) observed that the testis of the English sparrow increases in weight 192 times from the fall to the spring state. The data of Bergtold ('26) for maximal and minimal gonad weights of a great many birds show that the testis, even when larger than average, represents only $\frac{1}{57430}$ of the bird's body weight during the non-breeding season. During the mating period, however, it may represent $\frac{1}{19}$ of the body weight.

whereas juvenals of the same sex exhibit yellow or light horn-brown beaks. With the approach of spring, however, the beaks of juvenals turn black. Adult males may still further be distinguished from juvenals by the color of the feet; in the former they are black, in the latter a yellow color. Adults of both sexes may be distinguished from juvenals by the complete ossification of the skull. The skulls of juvenals are still soft in the fall of the year. Even if ossified in the region of the occipital bones, translucent 'windows,' or unossified areas, appear in the region of the frontal and parietal bones. Juvenal skulls become progressively ossified with the approach of spring. Adult birds may definitely be distinguished from juvenals until the latter part of January.

The testes of adult sparrows are somewhat larger than those of juvenals during the fall of the year. Among the juvenals variation in testis size is encountered, the gonads of birds of the first brood being slightly larger than those of subsequent ones. The testes of all males enlarge to mature breeding size in the spring.

Invariably the left testis is larger than the right. The following determinations are based exclusively on the left testis. The average juvenal testis length during October is approximately 1.7 mm.; diameter 1.2 mm.; volume 1.3 cu.mm.; weight 0.0019 gm. The testes of adult birds for the same time of the year average 2.62 mm. in length; diameter 2.09 mm.; volume 6 cu.mm.; weight 0.0061 gm. During April the testes of male birds weigh 0.3 gm. or more. The testis volume approaches 500 cu.mm.; length 11.5 mm.; diameter 9.0 mm. At this time of the year all birds are adults inasmuch as they are sexually mature and take part in mating activities. Bergtol ('26) reported the weight of the sparrow testis for February as 0.014 gm. This weight represented $\frac{1}{2050}$ of the body weight. In May the same author reported gonads weighing 445 mg. or $\frac{1}{57}$ of the body weight.

No noticeable increase in testis size of either juvenal or adult birds is evident until January. At this time, however, testes of both old and young males enlarge gradually. During

the month of January it becomes increasingly difficult to distinguish juvenal from adult birds because the beaks of the former are becoming black. Very few birds with yellow beaks are found during February. Birds with testes over 2 mm. in diameter invariably exhibit black beaks.

Marked increase in testis size is found about the middle of February. From March 4th to the 19th day of March inclusive an extreme increase in testis size can be noted. Mature sperms are found in the seminiferous tubules of the larger testes during the last days of the month, although maximum testis size has not been attained.

The changes in gross testis volume are presented in figure 1. Data on testis length, diameter and volume are given in table 1.

Microscopic studies of the testis tubules

The germinal epithelium of the juvenal testis contains only a single row of cells peripherally arranged near the basement membrane of the seminiferous tubules (fig. 5). Some cells possess a definite cytoplasm with round nuclei, whereas others exhibit a syncytial arrangement with elliptical or slightly irregular nuclei.

The adult testis presents a picture of inactivity during October (fig. 4). The seminiferous tubules are lined by large spermatogonia with round nuclei and definite cytoplasm. Cells with elliptical or slightly irregular nuclei, previously mentioned for the juvenal testis, are also present.

The tubules of both adult and juvenal testes are filled with a matrix in October. The matrix of the seminiferous tubules of the adult testis is not so regularly distributed as that of the young testis; it is interrupted here and there by spermatogonia which lie within the tubules. Cells with pyknotic nuclei are also found in the matrix (fig. 4). They are evidently undergoing degeneration.

The adult testis, during November and December, presents the same histological picture as that of October. Only spermatogonia and nuclei in syncytial arrangement are present in the germinal epithelium.

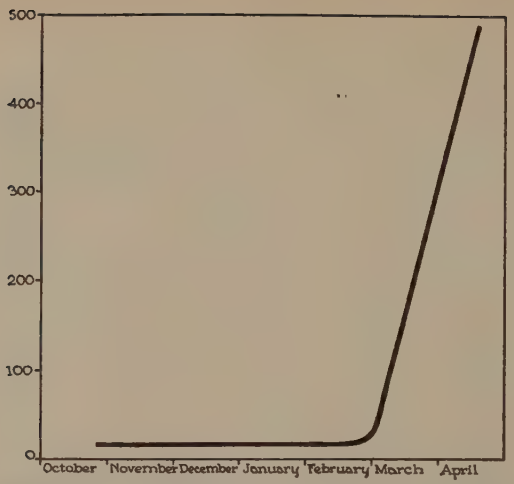

Fig. 1 Progression in testis volume from October 30th to April 15th. (Adults and maturing juvenals.) Volume is computed in cubic millimetres.

TABLE 1

Testis length, diameter and volume from October 4 to April 15

Date	Number of Birds	Length in mm. Mean	Length in mm. Extremes	Diameter in mm. Mean	Diameter in mm. Extremes	Mean Vol. in cu. mm.
			JUVENALS			
Oct. 4	1	1.26		1.08		.75
" 10	1	1.76		1.14		1.21
" 16	2	1.8	1.9	1.2	1.3	1.4
			1.7		1.1	
" 21	1	1.7		1.1		1.1
" 26	5	1.4	1.9	1.1	1.6	.9
			1.1		.8	
" 30	5	1.8	2.3	1.3	1.7	1.6
			1.7		.8	
Nov. 15	2	1.95	2.10	1.56	1.92	2.51
			1.80		1.20	
" 20	5	1.1	1.5	.9	1.1	.5
			1.2		.8	
Dec. 18	3	2.3	2.9	1.8	2.2	3.8
			1.9		1.4	
" 27	6	1.8	2.0	1.3	1.4	1.6
			1.7		1.2	
Jan. 8	1	1.8		1.6		2.4
" 15	5	2.3	2.5	1.8	2.1	3.9
			2.1		1.5	
			ADULTS			
Oct. 16	1	2.5		1.7		3.7
" 30	2	2.7	3.1	2.3	2.5	7.6
			2.3		2.1	
Nov. 20	1	2.2		1.7		6.7
Dec. 4	1	3.1		2.1		7.2
" 18	1	2.9		2.2		7.4
" 28	1	3.1		2.1		7.2
Jan. 2	2	2.3	2.5	1.9	2.1	4.2
			2.1		1.7	
			ADULTS AND MATURING JUVENALS			
Jan. 29	3	2.3	2.3	1.9	2.2	4.2
			2.3		1.7	
Feb. 19	6	3.3	4.5	2.3	3.2	9.1
			2.5		1.6	
" 25	5	3.8	6.0	2.95	4.5	17.9
			3.0		2.3	
Mar. 4	6	5.3	9.2	4.2	6.5	47.9
			2.5		1.8	
" 19	2	8.3	8.8	6.5	6.9	181.5
			7.7		6.0	
" 26	3	8.5	10.3	6.3	7.5	178.0
			6.3		4.5	
Apr. 2	5	10.5		8.0		354.0
" 15	5	11.5		9.0		487.9

As previously mentioned, all gonads show slight enlargement during January. Material collected January 15th indicates no formation of primary spermatocytes, but the germinal epithelium of birds collected January 29th shows a few spermatogonia which have pushed into the matrix of the seminiferous tubules; the chromatin of the nuclei of these cells is organizing itself into the heavy spireme characteristic of the primary spermatocyte.

During February the germinal epithelium indicates that true activity is imminent. The greatest degree of advancement over any previous stage is found in the largest testes obtained on February 12th (length 3.9 mm.; diameter 2.8 mm.). Great numbers of spermatogonia are within the seminiferous tubules; many are transforming into primary spermatocytes. There has been no spermatocyte division, however. Other testes obtained on the same date are not so large, and the germinal epithelium has not progressed beyond the condition found on January 29th.

The largest testis of February 19th (length 6 mm.; diameter 4.5 mm.) exhibits secondary spermatocytes and spermatids (fig. 6). The testes of most of the other birds shot the same day, however, have not reached division of the primary spermatocytes. Formation of primary spermatocytes and active mitotic division are under way when the testis assumes a length of 4 to 4.5 mm. and a diameter of 3 mm.

During the early part of March most testes become mitotically active. Of six males shot on March 4th, five present testes with spermatocytes or later stages in germ cell production. The largest testis shows a few metamorphosing spermatids. Thus, although active mitotic division may be observed as early as the middle of February, primary spermatocytes and later stages appear for the first time at the beginning of March. Testes for this period average 5.3 mm. in length; diameter 4.2 mm.; volume 47.9 cu.mm.

Metamorphosing sperms and a few spermatozoa may be observed in the gonads of birds collected on March 19th. However, a testis with great numbers of mature spermatozoa

is not observed until March 26th (fig. 7). Not all testes, however, are producing mature spermatozoa by March 26th. As far as the female is concerned, large follicles are found in the ovary at this time. The oviducts are hypertrophied. Not until April, however, do they appear ready to receive discharged egg cells.

Summarizing the above observations: The first signs of awakening to activity of the germinal epithelium in the male sparrow are apparent during the latter part of January. Marked activity, however, is not evident until the last days of February. During March the testes are in a state of active spermatogenesis. By the end of March mature sperms with heads embedded in the Sertoli cells are observed.

Experimental results

The effects of increasing the daily ration of light during October, November, December and January. Before exposing the birds to additional light histological examinations were made of the gonads of birds that had been confined to the control cage for a period of 2 weeks. Examination of the gonads revealed that they were in the resting condition. The inactive condition of the germinal epithelium is shown in figure 5.

Added light ration of approximately 6½ hours daily for a period of 6 weeks (during October, November and December) induces active spermatogenic development in both juvenal and adult male sparrows. The gross testis size reaches the proportions of normal testes for late March, and spermatogenically the testes of light treated birds are in the breeding condition. With increase in testis size there is corresponding development of the male secondary sex character of black beak by juvenal birds.

The response to increased daily light ration was probably no greater in December and January than in October, November and December. Although beaks of birds with testes over 2 mm. in diameter are always black in nature, this is not necessarily so experimentally. When the testis reaches full spermatogenic activity, however, the beak is usually jet black.

Twelve out of thirteen birds responded to added daily light ration, eleven of them showing marked testis enlargement. Data on the experimental birds appear in table 2. Figure 2 illustrates the change in testis size resulting from the light treatment. Figure 3 reveals the change in beak color as a result of testis enlargement. Figures 8 and 9 contrast the germinal epithelium of light-treated and control birds.

TABLE 2

Results of experiments in increased and decreased daily light ration

	Bird	Date Killed	Days of Added Light	Bill Color	Skull Ossification	Left Testis Length	Diam.	Vol.	Germ Cells
INCREASED DAILY LIGHT RATION	#1	Nov. 24	34	Gray	Juvenal	2.6mm.	2.2mm.	6.6cu.mm.	Many Spermatogonia
	#2	" 29	39	Black	"	5.9	4.8	71.2	Immature Sperms
	#2C	" 29	0	Yellow	"	1.7	1.2	1.3	Resting Spermatogonia
	#3	Dec. 4	44	Black	Adult	8.0	6.5	177.0	Mature Sperms
	#4	" 9	49	"	Juvenal	9.0	7.0	230.6	Mature Sperms
	#4C	" 10	0	Yellow	"	1.4	1.2	1.1	Resting Spermatogonia
	* #X	" 10	50	Gray	"	4.2	4.0	35.2	Spermatocytes
	#5	Dec. 14	54	Black	"	7.0	5.8	123.2	Mature Sperms
	#6	" 26	66	Black	"	7.5	6.7	176.1	Mature Sperms
	#6Ca	" 26	0	Yellow	"	2.0	1.5	2.4	Resting Spermatogonia
	#6Cb	" 26	0	"	"	2.1	1.4	2.2	Resting Spermatogonia
	#7	Jan. 4	29	Grey	"	6.0	4.5	63.7	Immature Sperms
	#8	" 7	32	Dark Gray	"	6.5	6.0	122.6	Mature Sperms
	#9	" 7	32	" "	"	6.0	6.0	113.1	Mature Sperms
	* #10	" 10	35	Yellow	"	Of juvenal size			
	#11	" 16	41	Black	"	7.8	7.0	200.3	Mature Sperms
	#12	" 16	41	Dark Gray	"	6.8	5.5	72.1	Immature Sperms
			Days of Decreased Light						
DECREASED DAILY LIGHT RATION	#13	Apr. 14	87	Yellow	Adult	2.2	1.9	4.1	Spermatogonia
	#14	" 14	87	"	"	2.4	1.8	4.1	Spermatogonia
	#15	" 14	87	"	"	2.4	1.9	4.5	Spermatogonia
	#16	" 14	87	"	"	2.5	1.9	4.7	Spermatogonia
	#17	" 14	87	Dark Gray	"	5.0	5.8	37.8	Spermatocytes
	#18	" 14	87	Grey-black	"	5.1	4.1	[illegible]	Spermatids
	#19	" 14	87	Black	"	6.7	5.1	91.2	Spermatids and some immature sperms
	#13C	" 14	0	"	"	7.0	5.5	110.9	Mature Sperms
	#14C	" 14	0	"	"	6.5	5.8	114.5	Mature Sperms
	#15C	" 14	0	"	"	8.0	7.0	205.3	Mature Sperms
	#16C	" 14	0	"	"	10.0	7.7	310.5	Mature Sperms

* (Sick - died)

Effects of decreasing the daily ration of light during January, February, March and April. Of seven birds kept on decreased daily light ration, none showed mature spermatozoa in April, the normal breeding season for the English sparrow, although the gonads of one bird, no. 19 (table 2), showed considerable increase in size. The gonads of birds no. 17 and no. 18 also increased in size considerably. Controls were at full spermatogenic activity. There is reason to believe that there are other factors operative beside light in producing the sexual cycle in view of the fact that there was active spermatogenesis in the testes of three birds kept

on decreased light ration. There were some spermatogonial mitoses in the testes of those birds in which there was practically no increase in gross testis size. The juvenals in which testis activity is suppressed retain light-colored beaks (see table 2 for data).

Fig. 2 Photograph showing results of experiment in increased daily light ration during October, November and December. 'Shot controls' (S.C.) are birds shot free in nature. The pair of testes labelled 'X' are those of an experimental bird which was killed December 11th. This particular bird had developed an infection and was on the point of death. The first pair of gonads in the EXP'L column represent the sizes of the testes when the experiment was begun on October 21st. Actual size.

DISCUSSION

The one ecological factor with which the normal enlargement and activity of the sparrow testis may be correlated most readily is the incidence of daylight. When temperature

for the year is lowest, January and February, the testis shows enlargement, and primary spermatocytes appear for the first time.

Food is not abundant at the time of year when enlargement of the testis begins. In earlier months food is more available, but enlargement does not take place. The work of Bissonnette ('32), however, has shown that the type of food is an important factor to be reckoned with in the sexual cycle of birds.

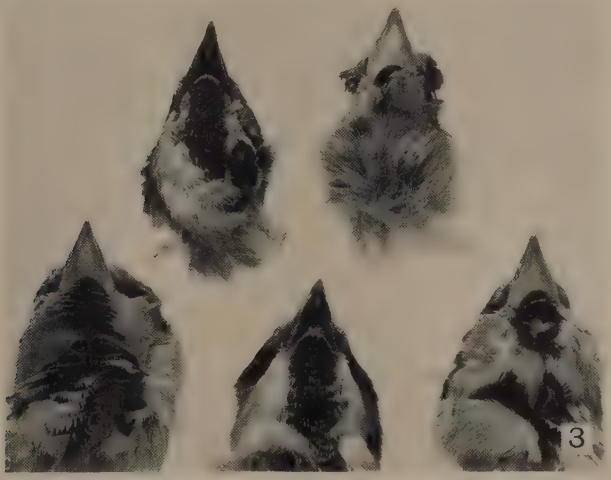

Fig. 3 Photograph showing change in beak color of juvenal birds placed on added daily light ration. The upper two heads are from experimental and control specimens killed December 9th and 10th. Those below are from an experimental and two controls killed December 26th. As the testes of experimentally activated juvenals enlarged, the beaks turned black. Control males (juvenals) retained yellow beaks. Actual size.

According to Loisel ('00), temperature is the factor responsible for the variation in testis size. Rowan states, however, that if this were true there would be considerable disparity in the annual period of enlargement since the temperatures vary from year to year. This is not the case, however, for Loisel himself showed that the cycle is regular. The present investigation indicates that in at least the case of

the male sparrow light, rather than temperature, is the major factor in the production of the sexual cycle.

Rowan felt that activity, due to increased hours of wakefulness, is responsible for the progessive changes produced experimentally. Bissonnette found that muscular activity can modify the responses to light, but concluded that light in itself supplies the stimulus for the increased gonadal activity.

That there is a 'natural reproductive rhythm' has been pointed out by Rowan. Certain birds migrate across the equator to regions of longer day length; the gonads reach the winter minimum, coming to breeding size again the next year. Bissonnette showed that experimentally, in the case of starlings, once the peak of spermatogenesis is reached regression occurs, even though the birds receive added light.

The incidence of daily light appears to be the most important external factor in effecting the sexual cycle. Food and activity may modify the response to light as a stimulus. The results of the present investigation indicate that spermatogenic activity cannot be wholly suppressed in the spring by decreasing daily light ration, although birds can be brought to full spermatogenic activity in the winter by increasing the daily light ration.

The effect of the testicular hormone on the secondary sex characters was interesting to note in both the normal and experimental cycles. With increase in testis size there is a corresponding development of the male secondary sex character of black beak by juvenal birds. Beak color in the sparrow is a sex character dependent on testicular hormone. Keck ('32 and '34) observed change in the color of the adult male beak 5 weeks after castration to the light horn-brown of the female beak. The black beak of the castrated birds could be restored by injection of male sex hormone. Bissonnette stated that change in beak color of the starling is probably produced by a change in pigmentation brought about by exposure to light rays, rather than to testicular hormone action.

That the anterior pituitary is the intrinsic gonad stimulating mechanism in the sexual cycle of the sparrow has been

clearly demonstrated by Witschi and Keck ('35). They found that extracts of the anterior lobe of horse pituitary and Antuitrin, when injected into the sparrow during the period of gonadal inactivity brought about enlargement and germ cell activity. The seasonal activity of the gonads is dependent on the seasonal activity of the pituitary.[3]

In the case of birds, where breeding activity is restricted to the spring and early summer, there seems to be no doubt that the incidence of daily light is of utmost importance in determining the season of mating activity. As far as mammals are concerned, however, although certain sexual cycles have been modified by controlling daily light ration, Johnson and Gann ('33), Wells ('35) and others found that light is not a major factor in producing the sexual cycle in the gopher. Wells states that some environmental factor affects the pituitary, causing the sex cycle, but that factor has not been determined.

Bissonnette ('32) found that the female ferret responds more readily than the male to light treatment. Oestrus was induced in the anoestrus female. Hill and Parkes ('33) observed that this reaction was inhibited by hypophysectomy, concluding that light treatment stimulates the anterior pituitary. Baker and Ranson ('32) observed that the female of the field mouse is chiefly affected.

Dempsey ('34) found that exposure to daylight is not necessary for the induction of oestrus in the guinea pig.

CONCLUSIONS

1. The English sparrow possesses a decided seasonal sexual cycle. Minimum gonad size is found during October, November and December. Active spermatogenesis has begun during the last days of February, and mature sperms are produced for the first time during the latter part of March.

2. Increased daily light ration in mid-winter induces increase in testis size in both adult and juvenal birds. Testes

[3] According to Miller ('35), hyperthyroidism, brought about by injections of a water solution of crystalline thyroxine into the breast muscle, accelerates the seasonal sex cycle of the male sparrow both at the beginning and end of the breeding season.

experimentally enlarged reach the gross proportions of normal testes for late March. Spermatogenically the testes are in spring breeding condition.

3. Testicular enlargement and spermatogenic activity are closely correlated with the incidence of daylight. In the normal sexual cycle in Minnesota the first signs of germ cell activity are evident when temperature for the year is lowest.

4. High indoor temperatures (12° to 23°C.) produce no noticeable changes in the resting male sparrow gonad over the same period of time that increased daily light ration has a marked effect.

5. Reduced light ration in the spring retards the development of the germinal epithelium of the sparrow testis. Since there is not a complete suppression of germ cell activity when birds are given very little daylight, it seems that there are factors beside light operative in producing the seasonal sexual cycle.

6. The juvenal male acquires a black beak during testis enlargement. If testis enlargement is suppressed in the spring, the juvenal retains a light-colored beak.

ADDENDA

After the manuscript had been prepared the authors' attention was called to the work of Ivanova, Sophie, Arch. f. exper. Path. u. Pharmakol., vol. 179, p. 349, 1935. It was indicated in this paper that light is effective through the skin rather than the eyes in the activation of the sparrow testis by added daily illumination.

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PLATE 1

EXPLANATION OF FIGURES

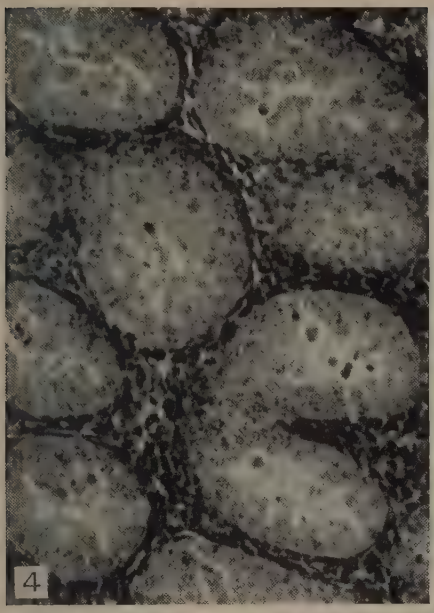
Sections cut at 10 μ

4 Portion of adult testis, October 30th. There are no mitotic figures. Stages beyond spermatogonia are absent. Normal bird killed free in nature. $\times$ 375.

5 Section of testis of juvenal bird killed October 21st, after 2 weeks of confinement. Inactive germinal epithelium. $\times$ 550.

6 Section of enlarging testis of sparrow shot February 19th. Primary spermatocytes are present; lumina appear in the tubules for the first time. Normal bird. $\times$ 375.

7 Section of testis exhibiting full spermatogenic activity, March 26th. Not all testis are producing mature sperms at this time. $\times$ 375.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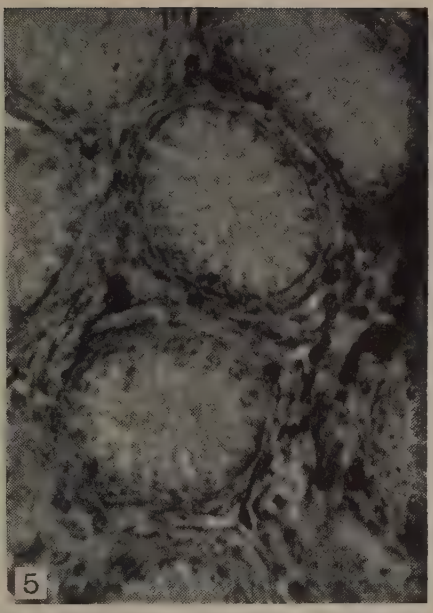
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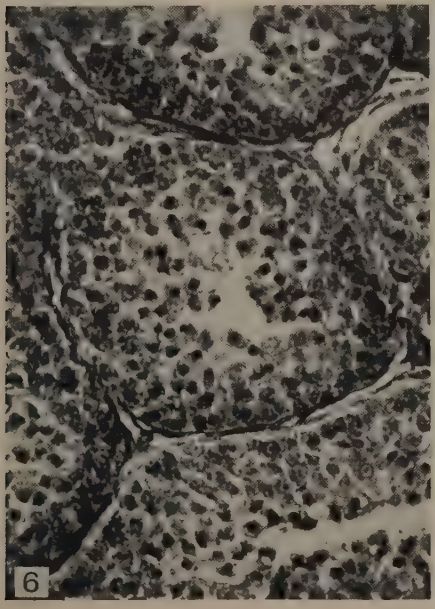
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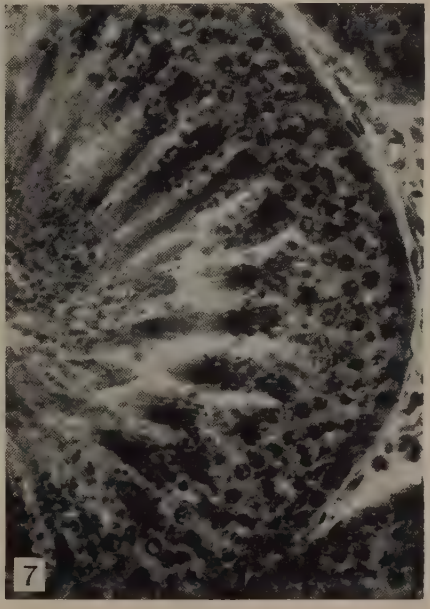
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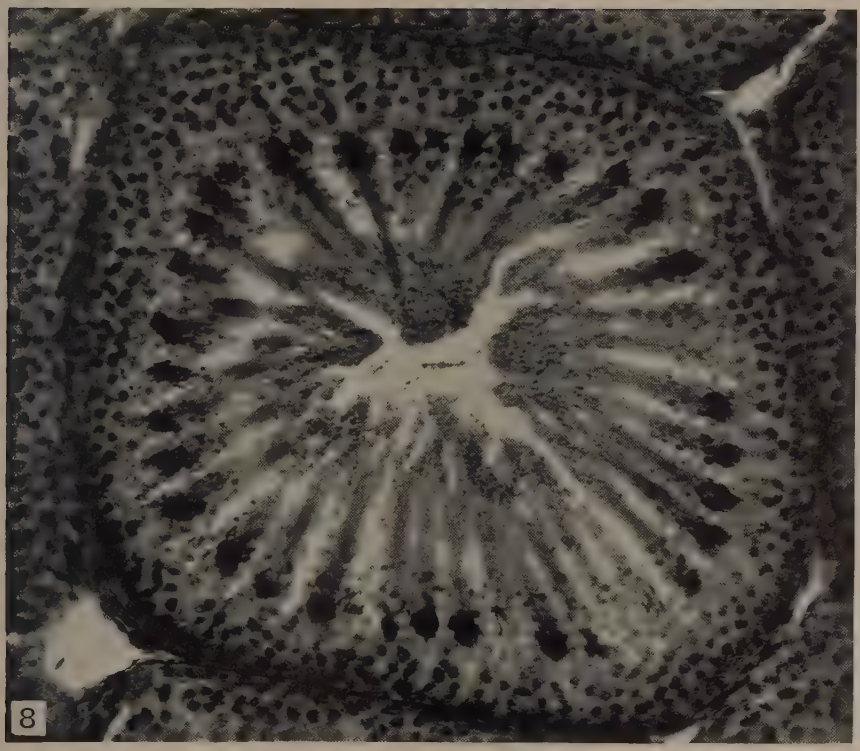
PLATE 2

EXPLANATION OF FIGURES

Sections cut at 10 μ

8 Section of testis of 'light bird' no. 6, killed December 26th. The bird had received increased daily light ration for 66 days. Note extreme spermatogenic activity. This condition is not normally attained until the latter part of March at the earliest. $\times$ 310.

9 Section of testis of 'control bird' no 6Ca. Killed December 26th after 80 days of confinement. Inactive germinal epithelium. $\times$ 310.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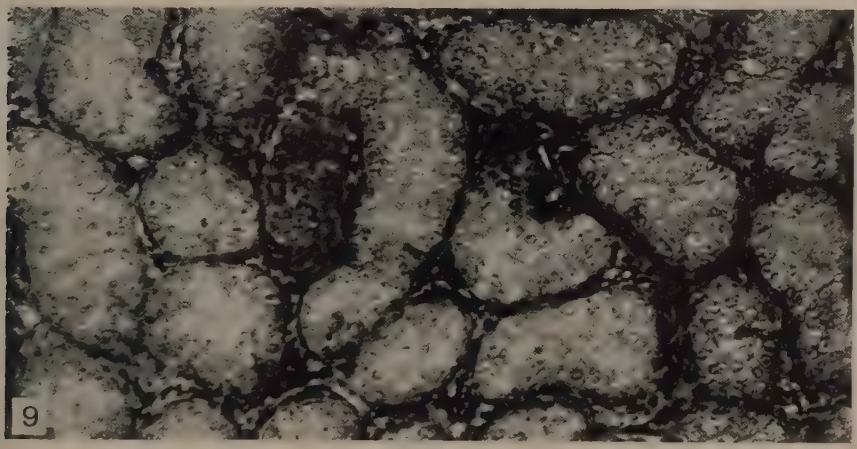
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EFFECTS OF OESTRIN INJECTIONS ON ACCESSORY REPRODUCTIVE ORGANS OF THE MALE GROUND SQUIRREL (CITELLUS TRIDECEMLINEATUS) [1]

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THREE PLATES (TWENTY-FOUR FIGURES)

INTRODUCTION AND PROCEDURES

In an earlier paper (Wells, '35) the writer described the seasonal changes in the reproductive system of the male ground squirrel and the general responses of the gonad and accessory reproductive organs to many different hormonal treatments such as those involved in castration, male hormone injections, administration of gonadotropic agents, and to other experimental manipulations (also see preliminary paper by Moore, Simmons, Wells, Zalesky and Nelson, '34). Whereas the accessory reproductive organs of the male responded during the season of low sexual activity to male hormone by showing rapid growth and intense secretory differentiation, following either the direct injection of male hormone or the stimulation of testicular activity by gonadotropic materials, it was learned very early in these experiments that the female hormone (oestrin) also caused a pronounced increase in the size of the male accessories. The investigation has involved the study of the reproductive system of more than 500 males, seventeen of which were injected with oestrin. The results of gross and histological studies of these responses form the body of this paper.

[1] This study was aided by a grant from the Rockefeller Foundation to The University of Chicago.

Seventeen male ground squirrels (seven adult and ten young) were given subcutaneous injections of oestrin, following the removal of one testis, epididymis and ductus deferens; the tissues removed at operation were studied both grossly and histologically, in order to determine the condition of the reproductive organs at the beginning of the experiment. The initial injection was given on the day of operation, except in the case of two 162-day bilateral castrates. Injections of either 12.5 or 25 rat units were administered daily for periods, ranging from 7 to 34 days, and autopsy occurred either during the season of low sexual activity or during the breeding period. At autopsy, all remaining reproductive organs were studied grossly and portions of each were prepared for histological examinations. Uninjected males, killed at all seasons of the year, furnished additional control data.

Details concerning collection, maintenance and care of the animals, routine autopsy procedure and the preparation of reproductive tissues for microscopical examination have been presented in the earlier papers. The writer is indebted to Dr. Carl R. Moore for general advice and to Dr. R. G. Gustavson of the Department of Chemistry at the University of Denver for the supply of oestrin, which was secured from extractions of human placentae.

NORMAL CONDITION AND EXPERIMENTAL RESPONSES OF ACCESSORY REPRODUCTIVE ORGANS

It should be recalled that the ground squirrel exhibits a single annual cycle of reproduction and that the male has fertile sexual capacity only during the spring breeding season. All accessory genital structures are in a functional mating condition during a short annual period, which is restricted to late April and May in most field animals; non-hibernating laboratory males attain breeding capacity as early as January and February but retain the characteristic annual cycle. Following the breeding season, all accessory reproductive structures undergo involution and remain essentially inactive for the greater portion of the year. Regression of acessory

organs is detectable by June 1st and has progressed to a definitive involution during the interval extending from July 15th to the latter part of August. Renewed activity in male accessories appears during the latter portion of the winter hibernation period and breeding capacity in the male is either present at the time of emergence from hibernation during the first 10 days of April or is acquired by the last week in April. Accessory organs of young males do not become functional until the spring following that of their birth.

1. Bulbar gland

The glandula bulbo-urethralis inferior is an unpaired cylindrical structure that is situated in a transverse plane between the base of the penis and musculus bulbocavernosus. According to Mossman, Lawlah and Bradley ('32), this gland is an expansion of Cowper's ducts; the fact (presented below) that both Cowper's ducts and the bulbar gland respond similarly to oestrin injections lends support to this contention. The bulbar gland is composed of a folded single-layered epithelium, and a coat consisting of a peripheral stratum of striated muscle and an inner layer of smooth muscle and connective tissue, which invades the central portion of the gland as trabeculae.

The average weight of the bulbar gland from nine normal males killed at the height of the breeding season was 0.9116 gm., while the average weight of bulbar from twenty-one animals killed at the period of low sexual activity was 0.1580 gm.—a ratio of 5.1 : 1 in seasonal range. In breeding males, the bulbar shows a greatly convoluted epithelium with granular pyramidal columnar cells and basal nuclei, inconspicuous lumina between the epithelial folds, a relatively scarce amount of smooth muscle and connective tissue in the trabeculae, and a thick layer of striated muscle in the peripheral coat (fig. 1). In animals sacrificed at the low point of reproductive activity, the gland exhibits a low epithelium with cells that have little or no distal cytoplasm and no cytoplasmic granules, relatively thick trabeculae of connective tissue and smooth muscle (there

being probably no actual hyperplasia beyond the condition found during the breeding period), large irregular lumina between the epithelial folds, and a thin layer of striated muscle in the coat (fig. 3). The bulbar gland of castrates resembles that of normal animals killed during the low period (compare figs. 3 and 5).

a. Oestrin injections for approximately 30 days in normal males during the period of low sexual activity and in castrates causes the bulbar gland to undergo gross distension accompanied by flaccidity, thus differing from the smaller rigid gland of uninjected controls. A yellowish fluid with the consistency of thick cream exudes when the peripheral coat of the gland is punctured; this fluid is peculiar to oestrin injected males since it is not found in normal untreated males at any stage of the seasonal cycle, and microscopical examination reveals it to be composed of granular secretion, leucocytes and desquamated cornified epithelial cells. The most conspicuous effect of oestrin on the bulbar gland is a metaplasia of the single-layered epithelium which becomes transformed into a stratified epithelium showing cornification. In animals receiving sufficient injections to elicit the greatest response, the bulbar epithelium seems to lack a basement membrane and presents the following microscopical appearance when observed from the basal portion toward the lumen: basal cells with basal nuclei, intercellular fibrils and intercellular bridges; cells showing axial orientation, intercellular fibrils and intercellular bridges, and lying in contact with the basal cells; a zone of large globular nuclei inside a syncytial cytoplasm, which stains rather deeply; large flattened cells, that contain either elongate or pycnotic nuclei, with distinct cells membranes and faintly staining cytoplasm; large cornified cells that are interspersed by polymorphonuclear leucocytes, but show no traces of nuclei; elongate strands of distorted cornified cells (compare figs. 4 and 6 with 3 and 5). Eleven daily injections produced a stratified epithelium of four to eight cells in thickness, but failed to induce cornification, the resulting epithelium strongly resembling the normal 'transitional type' found in

mammals. The bulbar gland of all males, injected for from 17 to 34 days, exhibited an epithelium of eight to fourteen cells in thickness, and quantities of cornified cells which were in a process of being sloughed into the lumina.

Problems of the origin of many layers within the metaplastic stratified epithelium are unsettled. It appears, however, that connective tissue cells may be transformed into epitheloid cells, since the basement membrane seems to be lacking and one can observe connective tissue cells aligned in a position so that a portion of their cytoplasm is within the epithelial layer and remaining portions appear extra-epithelial; no mitoses were observed.

Inter-epithelial connective tissue and smooth muscle were increased, beyond the relatively great amounts found in untreated controls, by oestrin administrations during the season of low sexual activity, but no mitoses of these cellular elements were detected. Large numbers of extravascular polynuclear leucocytes appeared throughout the bulbar gland of injected males, yet these leucocytes seemed to function principally in the dissolution of centrally located cells of the stratified epithelium and the resultant formation of large vacuolations.

b. Oestrin injections during the breeding season produced reduction in the gross size of the bulbar gland (of normal males) when it was compared with that of uninjected controls. The specific histological response to oestrin was masked by the simultaneous action of testicular hormone; however, metaplasia to stratified epithelium and mild cornification did occur (compare figs. 1 and 2).

2. Cowper's glands

The glandulae bulbo-urethrales from nine normal males sacrificed during the height of the mating season had an average weight of 0.3417 gm. while those from twenty-one animals killed during the low period averaged 0.0346 gm. (ratio of 9.8 : 1 in seasonal range).

In breeding males, the compound tubular Cowper's glands are composed of a folded epithelium containing a coarsely

granular cytoplasm (fig. 9). Peripheral tubules have a columnar epithelium with basal nuclei, while central tubules exhibit a cuboidal epithelium due to storage pressure of secretion in the lumina. Centrally located tubules are particularly distended by a granular secretion, which is apparently produced by merocrine erosion of the epithelial cytoplasm and which coagulates in the presence of air. Inter-tubular connective tissue and smooth muscle appear relatively scarce. Cowper's duct has a folded columnar epithelium with globular basal nuclei and a granular cytoplasm, and a lumen that is strutted with secretion. The wall of the duct contains an inner layer of smooth muscle and connective tissue, and an outer stratum of striated muscle.

At the period of low sexual activity, these glands show no folding of the low epithelium but usually contain a small amount of residual secretion in the lumina (fig. 7). This secretion is not being elaborated by the degenerate epithelium but exhibits distinct signs of destruction by vacuolization. Cells of the involuted epithelium have their cytoplasm reduced so greatly that its height is little more than that of the nuclei and its appearance agranular. Trabeculae of connective tissue and smooth muscle appear relatively conspicuous, yet this phenomenon is probably due merely to the regression of the epithelium and not to any actual increase in other cellular components. Cowper's duct retains very little residual secretion, and is composed of a low epithelium which contains very little agranular cytoplasm distal to the nuclei. The wall of the duct exhibits great reduction in the thickness of smooth and striated muscle layers; striated muscle fibers show morphological signs of involution, since the diameter of muscle fibers is only about half as great as that of those surrounding the duct in breeding animals.

a. Injections of oestrin into normal males and castrates during the season of low genital activity caused, without exception, increases in gross size of the Cowper's glands, thirty daily injections being sufficient to produce a glandular diameter that was approximately 100 per cent greater than

that for uninjected controls. The collecting portion of Cowper's gland and Cowper's duct responded histologically to oestrin in a fashion which was entirely similar to that described above for the bulbar gland. Here, as in the bulbar gland, the most conspicuous response resulted in a metaplastic stratification (compare figs. 7 and 8) and cornification of the epithelium, this phenomenon probably accounting for the greater portion of the gross increases.

The secretory portion of the glands responded to oestrin in an entirely different manner. Oestrin increased the height of such secretory epithelium and stimulated the appearance of granules within the cytoplasm. It should be stressed, however, that these granules differed from those found in normal males at any season of the year, since they were concentrated in clumps at the distal pole of epithelial cytoplasm. Furthermore, lumina of the tubules contained small quantities of secretion, which is atypical of that found in normal animals at any season by being interspersed with sloughed epithelial cells and leucocytes. In young males injected with 25 rat units daily for a relatively short period of from 7 to 17 days, the secretory epithelium exhibited signs of mild stimulation such as that found following the injection of small amounts of male hormone; other portions of the glands responded as in animals injected for longer intervals.

b. Oestrin injections during the breeding season decreased the gross size of Cowper's glands in normal males and greatly reduced the amount of secretion in the lumina. Collecting portions of the glands and the epithelium of Cowper's duct underwent the same type of metaplasia as was observed in the bulbar gland. Epithelium of secretory tubules was greatly decreased in height; but cytoplasmic granules failed to appear in clumps at the distal end of the cytoplasm, due possibly to the simultaneous action of male hormone produced within the organism.

3. Prostate gland

The glandula prostatica is an unpaired organ with two ventral prostatic ducts. The average weight of the prostate from nine breeding males was 0.2118 gm. while that for twenty-one normal animals killed during the low period was 0.0165 gm.—a ratio in seasonal range of 12.8 : 1.

In males at the height of reproductive activity, prostatic tubules are large and have prominent lumina; yet no secretion is stored in lumina of either secretory or collecting tubules. Secretory epithelium is greatly convoluted and is composed of columnar cells which have basal nuclei and a cytoplasm with no more than slight granulation (fig. 10). The epithelium of collecting tubules is either low cuboidal or at least somewhat lower than that of peripheral secretory tubules. Trabeculae of connective tissue and smooth muscle are limited to a very thin layer, which barely separates the epithelial basement membrane of one tubule from that of an adjacent one.

During the season of low sexual activity, the tubules are either small with inconspicuous lumina (fig. 11) or they lack lumina and show no other signs of epithelial organization. Erstwhile epithelial cells retain only traces of cytoplasm around their nuclei. Regression of tubular epithelium causes a relative conspicuousness of intertubular trabeculae which, at this season, appear as wide areas composed of connective tissue, smooth muscle and blood vessels.

a. Injections of oestrin in normal males and in castrates during the low period of genital activity increased the gross size of the prostate gland without exception; the diameter of the prostate in animals injected for 30 days was found to be approximately 100 per cent greater than that for untreated controls.

Histological responses were somewhat similar to those of the bulbar gland and Cowper's gland. Epithelium of the paired prostatic ducts underwent metaplastic stratification in every case, and in animals injected for intervals of 26 to 34 days it showed cornification. Oestrin induced stratification in the collecting tubules of the gland, epithelial thickness vary-

ing from two to ten cells; however, no cornification was observed (fig. 12): lumina of the collecting tubules contained great numbers of leucocytes and desquamated epithelial cells with pycnotic nuclei. The entire tissue of the prostate gland usually showed leucocytic invasion. The amount of inter-tubular connective tissue and smooth muscle was greatly increased by oestrin treatments.

The epithelium of secretory tubules responded to oestrin by the assumption of a peculiar morphology that consisted of a single layer of epithelial cells bordering the lumen and a concentric peripheral layer of cells; thus the epithelium of these tubules was given a double-layered appearance (fig. 12). The outer epithelial-like layer consisted of cells which were arranged more or less in a single row, and appeared to be possibly a product of inter-tubular connective tissue in the process of metaplastic transformation. The secretory epithelium in young males, receiving injections for periods of 7 to 17 days, exhibited a response similar to that induced by injections of male hormone, cytoplasm of the epithelial cells being increased in height; prostatic ducts of such animals showed metaplastic stratification of their epithelium.

b. Oestrin given to normal males *during the breeding season* caused both gross and hitological damage to the prostate gland. The diameter of the prostate from animals receiving twenty-six daily injections was approximately 60 per cent as great as that in untreated controls. Histological damage appeared to be largely restricted to reduction in the height of epithelial cytoplasm. Specific response by the prostate to oestrin administration consisted of stratification and cornification of the prostatic ducts; connective tissue, smooth muscle and tubular epithelium failed to show the responses which were described in animals injected during the low season, due probably to the simultaneous action of male hormone released by the testis.

4. Seminal vesicles

The vesiculae seminales removed from nine breeding males had an average weight of 1.0536 gm., while those secured

from twenty-one animals during the period of low sexual function showed an average weight of 0.0351 gm. The seasonal range in average weight may be expressed by the ratio of 30.0 : 1.

During the breeding period, seminal vesicles are composed of large tubules, the lumina of which are distended with great quantities of fine granular secretion that coagulates in the presence of air. Tubular epithelium consists of columnar cells that have basal nuclei and a great amount of distal cytoplasm (fig. 15). Circum-tubular smooth muscle and connective tissue are relatively scarce, due apparently to distention of tubular lumina by secretion and the resulting mutual pressure of tubules on adjacent ones.

At the season of low sexual activity, the seminal vesicles have small tubules that show no secretion in the diminutive lumina. Tubular epithelium is composed of cuboidal cells containing ovoid nuclei and little distal cytoplasm. The tubules are separated by relatively great amounts of connective tissue and smooth muscle (fig. 13), due probably to mechanical factors associated with epithelial regression and disappearance of stored secretion.

a. Administrations of oestrin to castrates and to normal males during the period of low sexual activity increased the gross size of seminal vesicles without exception, glands of animals receivings thirty daily injections weighing approximately five times as much as those from untreated controls. Glands from treated males were macroscopically more opaque and rigid than those from control animals. The ducts and the collecting regions of the glands were not studied histologically, since microscopical sections were prepared only from the secretory portions.

Prolonged injection periods of approximately 30 days resulted in the formation of whorls of connective tissue and smooth muscle which were far more conspicuous than those found in untreated controls (compare figs. 13 and 14). Connective tissue and smooth muscle seemed to exhibit both hyperplasia and cellular hypertrophy, but no mitoses were

observed; it should be stressed that direct objective measurement of connective tissue and smooth muscle stimulation was not employed. However, ocular micrometer measurements proved that the epithelium in glands from treated males was essentially similar to that of uninjected controls, thus offering indirect objective evidence that the gross hypertrophy of seminal vesicles was due to stimulation of connective tissue and smooth muscle. Both epithelium and intertubular tissue showed marked leucocytic invasion, while desquamated epithelial cells were occasionally found in the lumina of secretory tubules.

Oestrin injections for short periods of from 7 to 17 days in normal young males increased the height of epithelium in secretory tubules. This increase in epithelial height was due largely to a thickening of the cytoplasm, but secretion failed to be stored in the lumina.

b. Normal males injected during the breeding season showed great reduction in the size of their seminal vesicles which weighed only approximately 18 per cent as much as those from untreated control animals. Microscopical examinations revealed that diminution in size was due to decreases in the height of the secretory epithelium and conspicuous reductions in the amount of stored secretion within the lumina of the tubules. Stimulation of smooth muscle and connective tissue, if it occurred at all, was much less pronounced in males at this season than it was in animals injected during the period of low genital function.

5. Epididymis

The cauda epididymidis during the breeding period is very large in size and its tubular lumen is widely distended with spermatozoa. The coiled tubule is intimately surrounded by layers of circular and longitudinal smooth muscle; these layers are relatively thin at this season, due apparently to mechanical distension by spermatozoa in the lumen. Tubular epithelium is composed of a single layer of ciliated columnar cells, consisting of basal nuclei and much distal cytoplasm.

The amount of connective tissue between the closely packed coils of the tubule is relatively scarce (fig. 17).

At the season of low sexual function, the epididymis is very small and aspermatic. The coiled tubule possesses a tiny lumen and the tubular epithelium shows great cytoplasmic involution, so that epithelial cells lack cilia and are essentially cuboidal. Muscular coats appear relatively thick and coils of the tubule are separated by a relative abundance of connective tissue, due probably to shrinkage of the aspermatic tubule (figs. 16 and 19).

a. Oestrin injections into castrates and normal males during the season of low sexual activity significantly increased without exception the gross size of the epididymis. This gross reaction in animals receiving approximately thirty daily injections was found to be due to a great increase in the amount of intertubular connective tissue and a marked thickening of smooth muscle coats (compare fig. 20 with figs. 16 and 19); in such animals, the tubular epithelium lacked cilia, was essentially similar in height to that in uninjected controls, and spermatozoa were absent. Shorter intervals of injection varying from 7 to 17 days, resulted in less marked increases in connective tissue and smooth muscle, but apparently produced a mild stimulation of epithelial cytoplasm within the tubule.

b. Injections in normal males during the breeding season caused in every case gross reduction in the size of the epididymis. Factors responsible for this phenomenon appear to be both mechanical and hormonal, since spermatozoa were either absent or present in greatly reduced numbers and tubular epithelium showed histological signs of damage (compare figs. 17 and 18). Responses of connective tissue and smooth muscle to oestrin were either non-detectable or less noticeable than those in the epididymis of males, which were injected during the period of low sexual activity. Leucocytic invasion of all tissues within the epididymis was conspicuous.

6. Ductus deferens

The distal portion of this duct shows a seasonal variation in size, being approximately 0.4420 mm. in diameter in breeding males and about 0.1710 mm. in diameter from males killed during the period of low sexual activity.

At the height of reproductive activity, the epithelium consists of tall columnar cells which have basal nuclei and a great amount of distal cytoplasm bearing cilia. Coats of smooth muscle and circum-epithelial connective tissue are thick (fig. 21).

During the season of low sexual function, all tissue components of the ductus deferens undergo regression. Coats of connective tissue and smooth muscle are reduced in their absolute thickness. The epithelium becomes cuboidal and its cells show marked diminution of distal cytoplasm which lacks cilia (fig. 23).

Oestrin injections into castrates and normal males during the period of low sexual activity increased without exception the gross size of the ductus deferens; micrometer measurements showed that the diameter of the tube and thickness of the muscular coats were approximately 100 per cent greater than those in uninjected control animals. Histological study showed that this increased size was due largely to a pronounced increase in the amount of connective tissue and smooth muscle (compare figs. 23 and 24); apparently the cytoplasm of the tubular epithelium exhibited signs of stimulation. However, the degree of fixation shrinkage was not constant in the ductus deferens of all males, so that ocular micrometer measurements of the various layers of the tube were of relative rather than absolute value. Leucocytes were present in all tissues of the ductus deferens from each treated animal, while desquamated epithelial cells occasionally occluded the lumen of the tube.

In normal males injected during the spring mating period, the ductus deferens was found to be smaller than that in untreated controls; in such animals, the epithelium of the ductus deferens was lower than that in controls, while epithelial

cilia were either absent or comparatively inconspicuous. Connective tissue and smooth muscle coats in males treated at this season differed little, if any, from corresponding tissues in uninjected control animals (compare figs. 21 and 22).

DISCUSSION

Several investigators have reported an enlargement of secondary sexual organs in male mammals, following the addition of oestrogenic hormones, yet their interpretations of this response have resulted in a variety of diverging conclusions.

Houssay, Giush and Gonzales ('31) noted hypertrophy in seminal vesicles of male guinea pigs which bore ovarian grafts. They postulated that the ovarian graft possibly exerted its influence by stimulating the production of pituitary hormone, which acted on the testis, thus indirectly stimulating the seminal vesicles to their hypertrophic condition.

Seminal vesicle hypertrophy in rats, following oestrogenic administration, has been reported to be due to increases in smooth muscle (Freud, '33; Overholser and Nelson, '34, '35). Metaplasia of the seminal vesicle ducts of mice, in response to menformon injections, has been found by De Jongh ('35) who reports also a thickening of connective tissue and muscle in the coat of the ductus deferens.

Oestrogenic substances have been observed to induce metaplastic changes in the epithelium of the prostate gland and prostatic ducts in mice, these observations being made by La Cassagne and Villela ('33), Burrows and Kennaway ('34), Burrows ('35), De Jongh ('35), Weller, Overholser and Nelson ('36).

Prolonged injections of oestrin apparently have no direct effect upon the epithelium of either the prostate gland (Moore and Price, '31, '32) or the seminal vesicles of rats (Moore and Price, '31, '32; Overholser and Nelson, '34, '35), although oestrin indirectly reduces the epithelium of these glands.

Oestrin administration to either castrated or normal male ground squirrels during the season of low sexual activity induced gross hypertrophy of all accessory organs. Histological

analysis showed that this hypertrophy was due to either a metaplastic stratification and cornification of the glandular epithelium or a stimulation of smooth muscle and connective tissue. One concludes, therefore, that all this behavior is a specific response of accessories to oestrin and that it is entirely different from the response produced by male hormone. The apparent similarity of accessories from young males receiving oestrin for short periods to those from animals injected for short intervals with male hormone, presents a problem which has not been analyzed as yet. Oestrin injections into normal males during the breeding season produced gross and histological damage to all accessories; injections, given just previous to the peak of the mating period, prevented the normal development of accessory organs.

Some writers (Burrows, '35; De Jongh, '35; Freud, '33) observing metaplasia and increase in gross size of the prostate gland of castrated mice treated with oestrogenic substances, and the lack of such responses when male hormone was present, have compared the conditions with senile prostatic hypertrophy in man. It has been suggested that lowered production of male hormone in man may be accompanied by sufficient production of female hormone to cause prostatic hypertrophy, hence the therapeutic value of male hormone in man was suggested for such prostatic disorders.

In the ground squirrel, oestrin treatment of castrated males or normal males at low reproductive states resulted in great increases in all the accessory reproductive organs of the male, epithelial metaplasia or stimulation of muscle and connective tissue being involved. In contrast, similar dosages of oestrin during the breeding period, hence acting upon accessories that were influenced simultaneously by large quantities of male hormone, resulted in great decrease in the size of the organs and a markedly less degree of metaplasia and cornification. Whether this fact will have an intimate bearing on the prostatic problem in man, as suggested by others from observations on mice, must await further investigation.

SUMMARY

1. Oestrin injections during the period of low sexual activity produced gross hypertrophy of all accessory sexual organs, both in normal males and castrated animals. This stimulation, therefore, is induced by the direct action of oestrin on male accessories.

2. Oestrin produced a metaplastic stratification and cornification of the epithelium in bulbar gland, Cowper's glands (collecting tubules and Cowper's ducts) and prostatic ducts. Stratification of the epithelium with no cornification occurred in the collecting tubules of the prostate gland. The metaplastic epithelium resembled cutaneous epithelium in the possession of cytoplasmic bridges between the cells. Morphological evidence for apparent transformation of connective tissue elements into epithelial-like cells, in response to oestrin injections, was noted particularly in the secretory tubules of the prostate gland; evidence for such transformation in the metaplastic epithelium of the bulbar gland, Cowper's gland, Cowper's ducts and prostatic ducts was apparent but less striking than that mentioned above.

3. Oestrin increased the amount of connective tissue and smooth muscle in seminal vesicles, epididymis and ductus deferens.

4. Oestrin injections during the breeding season greatly damaged all accessory organs, specific responses to oestrin being less noticeable than in animals receiving treatment at other seasons.

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PLATE 1

EXPLANATION OF FIGURES

(All sections cut at 4 μ and magnified $\times$ 150)

1 Bulbar gland of normal breeding male, killed 4-29-33.

2 Bulbar gland of injected male. Injected daily during the breeding season with 25 R.U. of oestrin for 26 days and killed 4-29-33.

3 Bulbar gland of normal male, killed during the period of low sexual activity on 11-19-33.

4 Bulbar gland of injected male. Injected daily with 25 R.U. of oestrin for 29 days and killed during the period of low sexual activity on 11-6-32.

5 Bulbar gland of untreated 162-day castrate, killed 11-6-32.

6 Bulbar gland of injected 162-day castrate. Injected daily with 25 R.U. of oestrin for 29 days and killed 11-6-32.

7 Cowper's gland of normal male, killed during the period of low sexual activity on 11-19-33.

8 Cowper's gland of injected male. Injected daily with 25 R.U. of oestrin for 29 days and killed during the season of low genital activity on 11-6-32.

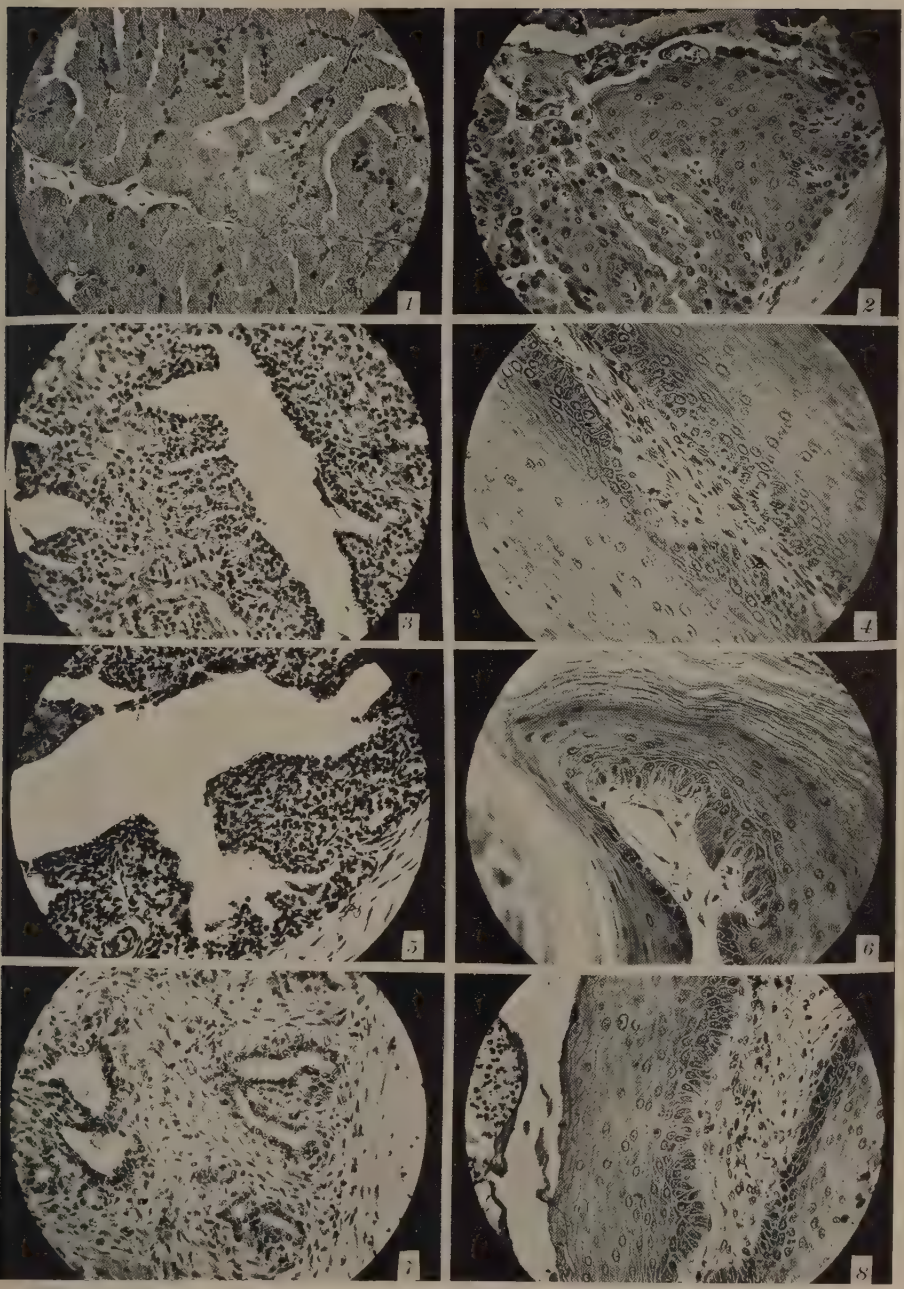
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PLATE 2

EXPLANATION OF FIGURES

(All sections cut at 4 μ and magnified $\times$ 150)

9 Cowper's gland of normal breeding male, killed 4-29-33.

10 Prostate gland of normal breeding male, killed 4-29-33.

11 Prostate gland of normal male, killed during the period of low sexual activity on 11-19-33.

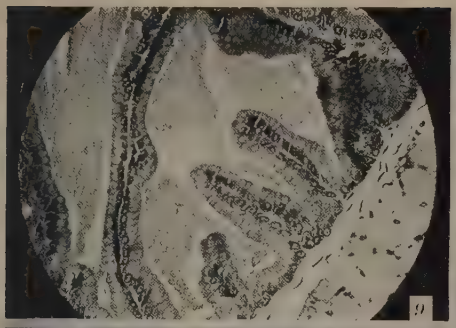
12 Prostate gland of injected male. Injected daily with 25 R.U. of oestrin for 29 days and killed during the season of low sexual activity on 11-6-32.

13 Seminal vesicle of normal male, killed during the period of low sexual activity on 11-19-33.

14 Seminal vesicle of injected male. Injected daily with 25 R.U. of oestrin for 29 days and killed during the period of low sexual activity on 11-6-32.

15 Seminal vesicle of normal breeding male, killed 4-29-33.

16 Epididymis of normal male, killed during the season of low sexual activity on 11-19-33.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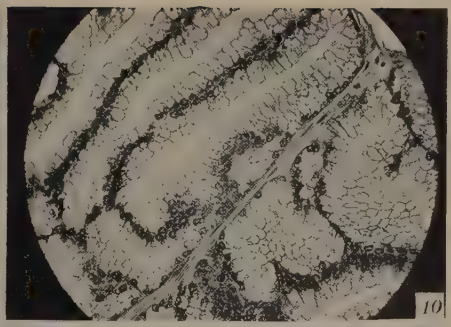
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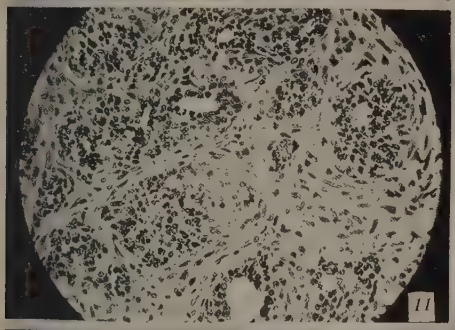
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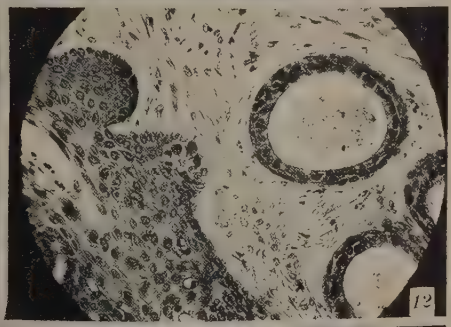
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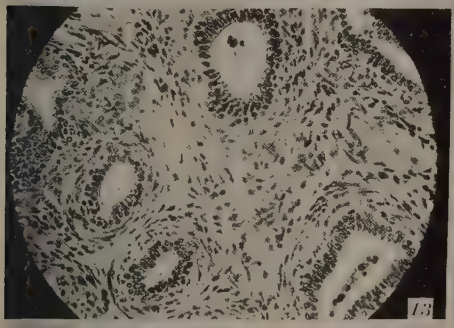
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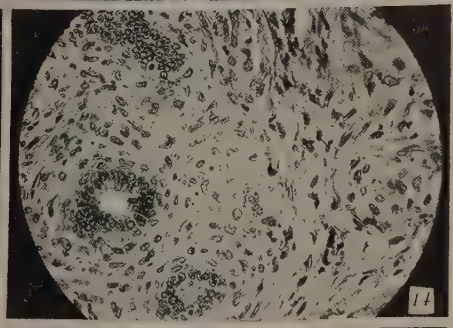
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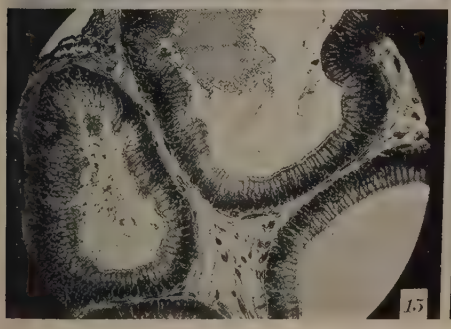
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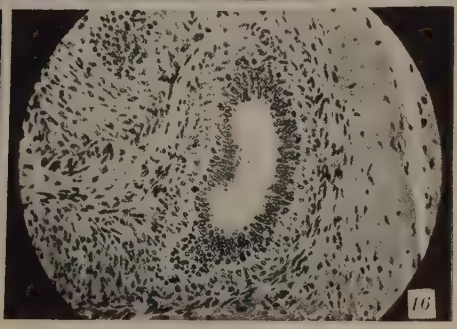
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PLATE 3

EXPLANATION OF FIGURES

(All sections cut at 4 μ and magnified $\times$ 150)

17 Epididymis of normal breeding male, killed 4-29-33.

18 Epididymis of injected male. Injected daily during the breeding season with 25 R.U. of oestrin for 26 days and killed 4-29-33.

19 Untreated epididymis of an injected male (animal 345). Epididymis surgically removed, immediately preceding the initial injection, during the season of low sexual activity.

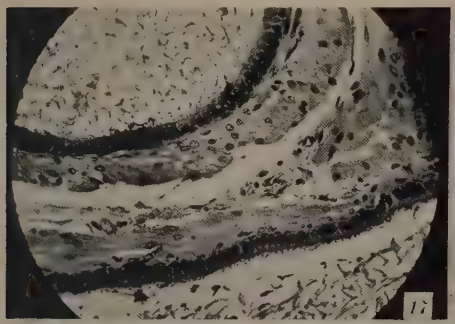
20 Autopsy epididymis of an injected male (animal 345). Animal injected daily with 25 R.U. of oestrin for 29 days and killed during the period of low sexual activity on 11-6-32.

21 Ductus deferens of normal breeding male, killed 4-29-33.

22 Ductus deferens of injected male. Animal injected daily during the breeding season with 25 R.U. of oestrin for 26 days and killed 4-29-33.

23 Untreated ductus deferens of injected male (animal 345). Ductus deferens surgically removed, immediately preceding the initial injections, during the season of low sexual activity.

24 Autopsy ductus deferens of injected male (animal 345). Animal injected daily with 25 R.U. of oestrin for 29 days and killed during the period of low sexual activity on 11-6-32.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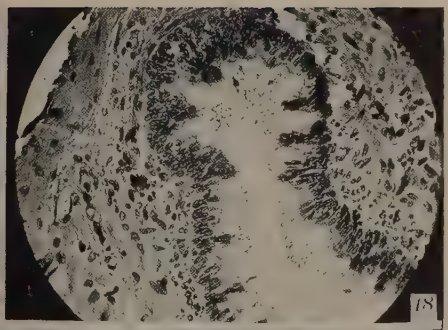
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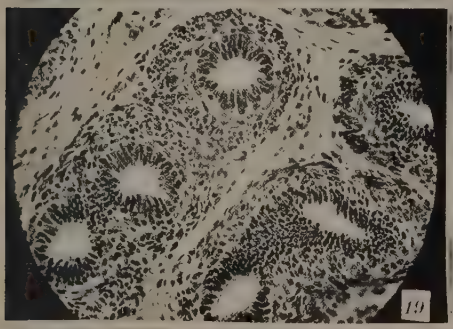
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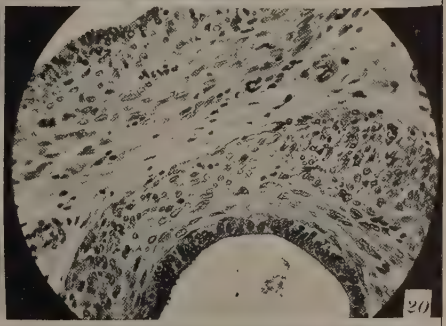
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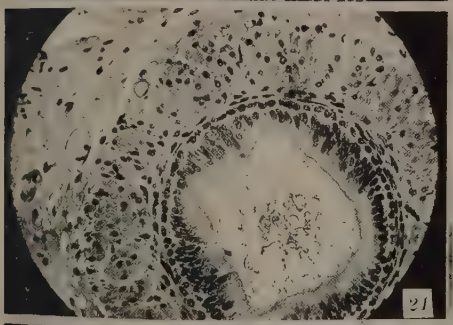
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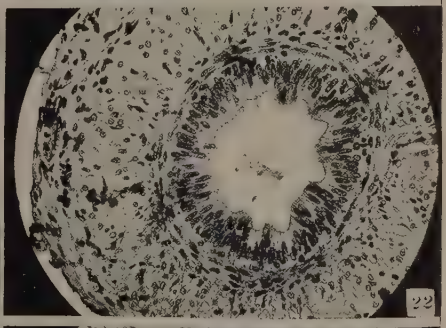
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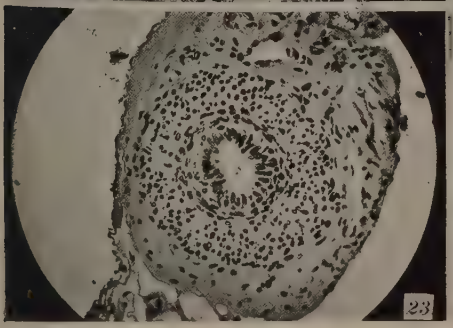
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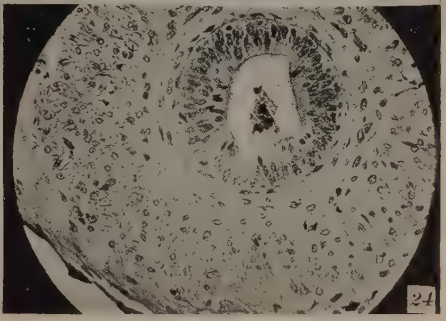
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A METHOD OF ILLUMINATING LIVING STRUCTURES FOR MICROSCOPIC STUDY [1]

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Hull Laboratory of Anatomy, The University of Chicago

FIVE FIGURES

INTRODUCTION

This paper discusses the present stage of development of a method of illuminating living tissues, especially the internal organs of small animals, for microscopic study. The method was designed at first to study the mesonephros of the frog. However, it soon became apparent that it could be applied to the study of many other structures. Thus it became necessary to design apparatus and procedures of general applicability so that special modifications of the general method could be used to study various tissues and organs. It has been an ideal that the method should enable us to study living organs under circumstances that very closely approach normal physiological conditions. I recognize clearly that this ideal has not been fully attained; this method in its present stage of development is only a step in that direction.

The bare essentials of the method have already been described (Knisely, '34).

This presentation discusses some of the individual problems that have been encountered in developing the method. It is

[1] This work was aided by a grant from the Rockefeller Foundation to the Biological Sciences Division of The University of Chicago.

This method has been developed under the direction of Dr. R. R. Bensley. The assistance and counsel of Doctor Bensley and the other members of the staff of the Hull Anatomical Laboratory have been invaluable in this work.

The method was demonstrated at the 1934 meeting of the American Association of Anatomists, at the University of Pennsylvania.

obvious that to some problems there are somewhat satisfactory solutions, to others partial solutions, but to still others only unsatisfactory compromises with difficult situations. I choose to discuss the individual problems of the method, so that each can be recognized and perceived to be an entity, and so that the present effort toward a solution may be recognized as a solution, a partial solution or a compromise. I hope by thus subdividing the discussion to facilitate new and better efforts toward solving these problems.

These are not new problems. There is little, if anything, new in the individual partial solutions. It is rather the combination of these solutions and compromises in a particular manner, that makes the method useful.

THE PROBLEMS

1. ADEQUATE ILLUMINATION OF RELATIVELY INACCESSIBLE STRUCTURES, USING A MINIMUM OF SURGICAL PROCEDURE

The method of illuminating living tissues depends upon and is built around the properties of fused quartz.[2] Light is conducted to the exact area to be studied by means of a suitably shaped rod of fused quartz.

Most of a beam of light falling upon the base of a clean, smooth, polished rod of this material is reflected back and forth inside the rod; much of the light is retained within the rod until it reaches the opposite end. This is true even though there are several bends and a 'concentrating segment' in the rod. This property of conducting light somewhat like a hose conducts water is usually termed 'internal reflection.' Various internal reflection systems have been used to illuminate objects for microscopic study.

Basler ('17) was the first to design a method of conducting light to living tissue by means of internal reflection. He used a curved rod of glass. Loeffler and Nordman ('25) used Basler's method in their study of the liver circulation.

[2] The terms 'fused quartz' and 'vitreous silica' indicate one material. The term 'quartz' which is often loosely applied to fused quartz should be reserved to designate crystalline quartz. The optical properties of crystalline quartz and fused quartz are not the same.

Basler's description of his method includes an accurate comprehensive mathematical description of the phenomenon of internal reflection. His equations apply to fused quartz as well as to glass, provided one substitutes in the equations the indices of refraction and critical angles of fused quartz. Sosman, in his 1927 monograph, The Properties of Silica, gives tables of the indices of refraction of fused quartz for many wave lengths of the visible spectrum. The corresponding critical angles can be calculated from the equation $\frac{1}{\text{index of refraction}} =$ the sine of the critical angle.

Fused quartz is superior to glass for conducting light for four reasons: 1) Fused quartz is much more transparent than glass. Glass absorbs a considerable proportion of the light entering it. 2) Glass does not absorb all wave lengths equally so that a glass rod delivers light of a color different than that sent into it. Fused quartz is equally transparent to all wave lengths of the visible spectrum and so delivers light of the same composition that it receives. 3) A great deal of light is lost from the sides of a glass rod by lateral refraction. 4) In order that light may be internally reflected around curves in a glass rod, they must be of long radius. Light is not reflected around curves of short radius in glass rods in usable amounts.

Florey and Carlton ('26) spread the mesentery out over a glass plate and illuminated the area to be studied by means of an internal reflecting rod. They do not name the material of which the rod was made.

Leiter ('25) used a curved, fused quartz rod to illuminate microscopic work, because the passage of light through the rod eliminated the image of the lamp filament from that of the specimen.

Silverman ('25) described the use of curved fused quartz rods to furnish 'cold light'[3] for microscope use.

Wearn, et al. ('34), in the study of the circulation of the living mammalian lung, used a straight truncated cone of fused quartz to conduct light to the lung tissue.

[3] The term 'cold light' is misleading. A fraction of the light energy which strikes an object is transformed into heat.

Because light is internally reflected around long curves and sharp bends in a smooth, clean, polished rod of fused quartz, a rod can be so shaped that light can be conducted to almost any place in an animal's body. By use of a suitable primary light source and a 'concentrating segment' in the rod, nearly any place in a small animal's body can be brilliantly illuminated.

Control of brightness of illumination is inexpensively secured by means of a break in the fused quartz rod (at L in fig. 5). By placing the segments of the rod more or less out of alignment with each other, the amount of light entering the distal segment is controlled.

Light filters are inserted in the break in the rod if in a particular experiment colored light aids in differentiation.

By adjusting the position of the delivery tip, a structure may be seen by reflected light, 'low incident light' (compare Vonwiller), or by transillumination.

2. TEMPERATURE CONTROL

It is essential that the experimental procedures employed, insofar as possible, do not cause, and do not fail to prevent, alterations in the temperature of the structures being studied or in the temperature of the whole animal.

1. Control of the temperature of the tissue being studied

a. Conduction. Fused quartz is a very poor conductor of heat. Its thermal conductivity factor at room temperatures is about 0.003,[4] according to Sosman. Owing to the low thermal conductivity of fused quartz plus the break in the rod, heat from the light source is prevented from reaching the tissue by conduction. And, being such a poor heat conductor, the fused quartz rod does not conduct heat away from mammalian tissues rapidly enough to lower the tissue temperature appreciably.

[4] That is, 0.003 20°-gram-calories per square centimeter per second, per degree Centigrade per centimeter.

b. Radiation. Two steps are taken to protect the tissue from the heat developed by transformation of radiant energy. First, the quantity of light reaching the tissue is kept at a minimum, and second, the heat which is produced by transformation of radiant energy is removed as fast as it is produced, either at, or but slightly above, the normal temperature of the tissue being studied.

To keep the quantity of radiant energy reaching the tissue at a minimum, three preventive measures are taken. 1) The dimmest illumination by means of which one can see clearly is used. 2) A filter (at L in fig. 5) removes wave lengths not desired in a particular experiment. 3) Not much more tissue is illuminated than can be studied at one time.

To remove the heat developed by transformation of radiant energy, at the temperature of the tissue, as fast as it is produced, a *flowing* wash solution of constant temperature is applied to the exposed tissue by means of two glass tubes (g and g in fig. 5). One of the tubes is fastened to the fused quartz rod by means of small rubber bands. It always accompanies the rod. The flow from this tube is directed across the surface of the rod's delivery tip so that there is a constant flow of fluid between the tissue and the tip of the rod. The fluid from the other glass tube is directed over the rest of the exposed surface of the organ being studied, and across the surfaces of other exposed structures.

For amphibian experiments, the solution is at room temperature. For mammalian experiments, a 10-liter stock bottle full of fluid is kept in a constant temperature chamber, at a temperature high enough so that the temperature of the fluid as it leaves the tips of the glass tubes is just the normal temperature of the organ being studied.

It is essential that the rate at which the wash solutions leave the glass tubes be carefully controlled, for if the flow is too slow the heat developed is not carried away as fast as it is produced and so the temperature of the tissue rises; and if the flow is too rapid the beating of the wash solution against the tissue frequently initiates a hyperemia. The optimum rate is just fast enough to keep the tissues moistened well.

When working with a water immersion lens I usually place the upper glass tube tip against the side of the end of the objective and let the fluid flow gently down across the objective onto the tissue. The force of the stream is thus spent against the side of the objective rather than against the tissue. And the film of wash solution which is maintained between objective lens and tissues acts as immersion fluid.

2. Control of the temperature of the bodies of mammals

The bodies of mammals are blanketed with dry cotton to keep them warm. Wicks of absorbent cotton conduct the used wash solution into the drip tray, so preventing the spent wash solution from wetting the cotton blanketing.

Because fused quartz is highly transparent to the visible spectrum, light can be conducted through long rods (30 to 75 cm. long, in practice). This permits the hot light source to be far enough from the animal so as not to affect it.

3. PREVENTION OF EFFECTS OF RINGER'S SOLUTION

Amphibian Ringer's solution in contact with an amphibian's skin affects the animal's water balance which in turn affects some circulatory processes. To prevent spent wash solution from affecting an amphibian, as it drains away from the site of an incision, a gentle flow of plain water is directed against the animal's skin so that the waste Ringer's is greatly diluted at the animal's surface.

4. DELIVERY OF INTENSE LIGHT INTO THE BASE OF A ROD

It is necessary, for some observations, to be able to send very bright light into the base of a rod. There are two factors in the problem of getting enough light into a rod: 1) the choice of a primary light source, and 2) collecting the light from the source and sending it into the rod.

1. The primary light source

It is desirable to have as bright a light source as possible, and it is desirable that the source be such that one can get the

base of the rod very close to it. It would seem that a naked electric carbon arc answers these two conditions. But carbon vapor from the arc tends to sublime on the base of the rod, so preventing light from entering it. Also inexpensive carbon arcs require frequent adjustment and they produce ultraviolet light. (Campbell and Hill, '24, demonstrated that ultraviolet light from carbon arcs 60 cm. from the tissue produce pathological dilatation and stasis in capillaries.) Fused quartz rods will conduct ultraviolet light. For these reasons carbon arcs are not used.

Tungsten filament motion picture projection bulbs are used as primary light sources. According to Coblenz ('30), tungsten filaments in glass bulbs emit very little ultraviolet light beyond 320 μμ. In use at present are 200-watt and 400-watt 'T 10' bulbs, and 500-watt and 1000-watt 'T 20' bulbs.[5] (By definition a 'T 10' bulb is 10/8 inches (31.75 mm.) in diameter; a 'T 20' is 20/8 inches (63.5 mm.) in diameter.)

The life of these bulbs can be greatly increased by adequate cooling. To cool a bulb it is enclosed in a stove pipe through which air circulates. The 200-watt 'T 10' bulb is enclosed in a 3-inch (7.5 cm.) stove pipe 24 inches (60 cm.) high, and cools itself by convection. The 400-watt 'T 10' bulb is enclosed in a 3-inch (7.5 cm.) pipe and cooled with a fan (fig. 5). The 500-watt and 1000-watt bulbs are enclosed in 6-inch (15 cm.) pipes and cooled by means of fans.

Infra red light. The fused quartz itself absorbs certain bands of the infra red. Sosman ('27, p. 719) says, "In the ultra red spectrum quartz (crystalline quartz) shows decided absorbing power at several frequencies, notably at about 3 μ, 4 μ, 5.3 μ, and between 6 and 7 μ. Strong reflection then follows at about 9 μ, 13 μ and 21 μ." (See table XXXV in Sosman.) "Vitreous silica (i.e., fused quartz) absorbs at about the same frequencies as quartz but to a smaller degree."

[5] These are Mazda bulbs, and can be obtained from General Electric Company or from Westinghouse Lamp Company, both at 20 North Wacker Drive, Chicago, Illinois.

Special precautions to remove the unabsorbed infra red have not been taken so far. If complete removal of the infra red proves to be desirable, filters can be inserted in the break in the rod or other methods used to eliminate it.

2. Collecting light and sending it into the rod

The base of the fused quartz rod is placed against the bulb directly opposite the tungsten filament (fig. 1). The base of the rod is only $\frac{5}{8}$ of an inch (16 mm.) from the filament of a 'T 10' bulb; $1\frac{1}{4}$ inches (32 mm.) from the filament of a 'T 20' bulb. The bulbs are set in prefocused sockets so that the

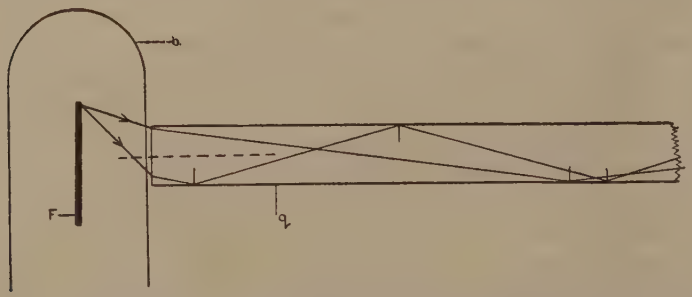


Fig. 1 Shows path of two rays of light from one end of filament (F) as they pass through glass bulb (b) into fused quartz rod (q). Note that each ray is refracted as it enters the rod so that it becomes more nearly parallel to the rod's axis.

plane of the filament is maintained approximately perpendicular to the long axis of the rod. A ray of light, which falls on the base of the rod is bent 'toward the perpendicular' (fig. 1); that is, toward the axis of the rod. This makes most rays in the rod strike the inside surface at an angle greater than the crtical angle of fused quartz and so they are reflected back into the rod again.

There are three reasons why a condenser lens system is undesirable in this work: a) A considerable portion of the light which falls on the receiving side of a condenser system is 'absorbed' (that is, transformed into heat) and so is not available for illumination. b) Since inexpensive condenser

systems are not corrected for spherical aberration, they do not direct the light to a single small point but spread it over quite an area, even when carefully focused. Thus much of the light transmitted by the condenser cannot be sent into the base of a fused quartz rod. The third objection is most important. c) In order to focus a simple condenser system, the lens of the system which receives the light must be placed as far from the light source as the first focal length of the first lens of the condenser. This distance is so great that (owing to the fact that the intensity of illumination of a surface is inversely proportional to the square of the distance from the

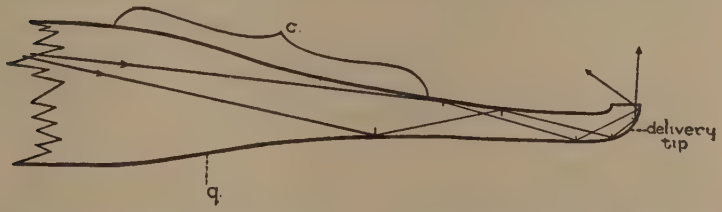


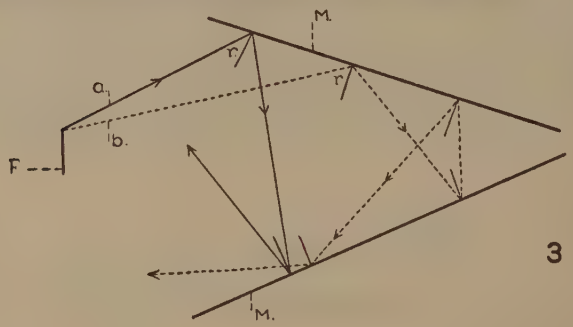
Fig. 2 One form of light delivery tip of a fused quartz rod. Two rays of light are shown. Note that they leave the tip at different angles. The light from the tip is diffused, not focused. The bracket c marks the position of a 'concentrating segment.'

The delivery tip can be made in almost any shape that is needed to conduct light to a particular place. The emitting surface of the delivery tip can be ground (on an oil stone) or fire-polishd as desired.

light source to the surface) the inexpensive condenser systems available do not collect sufficient light energy for the work. These three factors operating simultaneously greatly reduce a condenser system's efficiency. When, for some special purpose particularly intense illumination is desired, a collecting device is needed which will absorb little light, which will direct nearly all of the light collected into the rod, and which has a very short first focal length so that it can be placed close to the light source and collect illumination of high intensity.

While I was studying the conditions of the problem it seemed that perhaps a hollow truncated, cone-shaped, silvered reflector would serve the purpose. Consider the sagittal

section of a truncated cone represented in figure 3. A ray of light (a) striking one side of the cone is reflected twice and then passes out the base of the cone again. Ray (b) is reflected four times before passing back out again. It becomes apparent that but a rather small fraction of the light entering the base of such a cone passes out the small end.



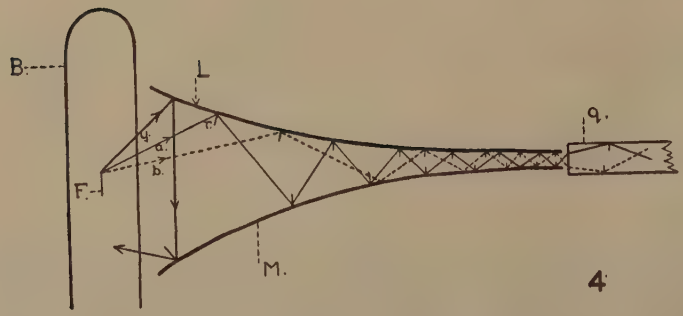


Fig. 3 A sagittal section of a hollow truncated cone having a surface (M) which reflects the light. Rays of light (a and b) from filament (F) are reflected several times and pass back out the large end of the cone. The cone does not act as an efficient light-collecting surface.

Fig. 4 The 'concentrating segment.' Showing a reflecting surface that does act as a light-collecting surface. (F) is the filament in bulb (B). *M* is the mirrored light-collecting surface shown in sagittal section. The ray (y) is reflected twice and then passes out the large end of the concentrating segment. The rays *a* and *b* fall on the surface behind the limiting position (L) and are reflected again and again until they pass out the opposite end and into the rod (q).

If a truncated cone depends upon 'internal reflection' rather than reflection by a silvered surface, then at whatever point the angle between the ray of light and the perpendicular becomes less than the critical angle of internal reflection between the two media, the ray is not reflected in again but passes out of the side of the cone. After each reflection the incident angle becomes smaller so that ultimately a great deal of the entering light is lost by lateral refraction.

But consider a simple modification of the truncated cone, a sagittal section of which is shown in figure 4. A ray of light striking such a curved surface is reflected in such a way that after each reflection the ray becomes more nearly parallel to the axis of the surface; much of the light entering the large end of this surface is reflected back and forth until it passes out the small end.[6] Such surfaces have been made, some by drawing out hot thin glass tubes of large diameter, and mirror-silvering the outside of the glass, some by drawing out fused quartz rods (fig. 2). They work very well as light collecting and concentrating instruments. The afferent end can be made as large as one needs and the efferent end may be made very small to concentrate the light. They do not absorb a great deal of the light energy. The large end may be placed as close to the light source as one desires. The term 'concentrating segment' is used to indicate a surface of this general shape, whether it is a silvered glass surface or the surface of a fused quartz structure. The light coming from the small end of a concentrating segment is diffused, not brought to a pencil focus like the light coming from a lens.

A silvered glass concentrating segment is not shown in figure 5 between the light bulb and base of the fused quartz rod. If especially intense illumination is needed one can be inserted. A concentrating segment in the fused quartz rod (fig. 2) concentrates the light in the rod into the delivery tip.

[6] It was thought that perhaps a mathematical analysis of the curve of this surface might indicate some modification which would increase its efficiency. Several mathematicians have worked upon the problem, each concluding that any empirical curve approximating the one depicted in figure 4 will serve this function efficiently enough for our purpose and that the expense of making more accurate ones would be greater than the value of the increased efficiency.

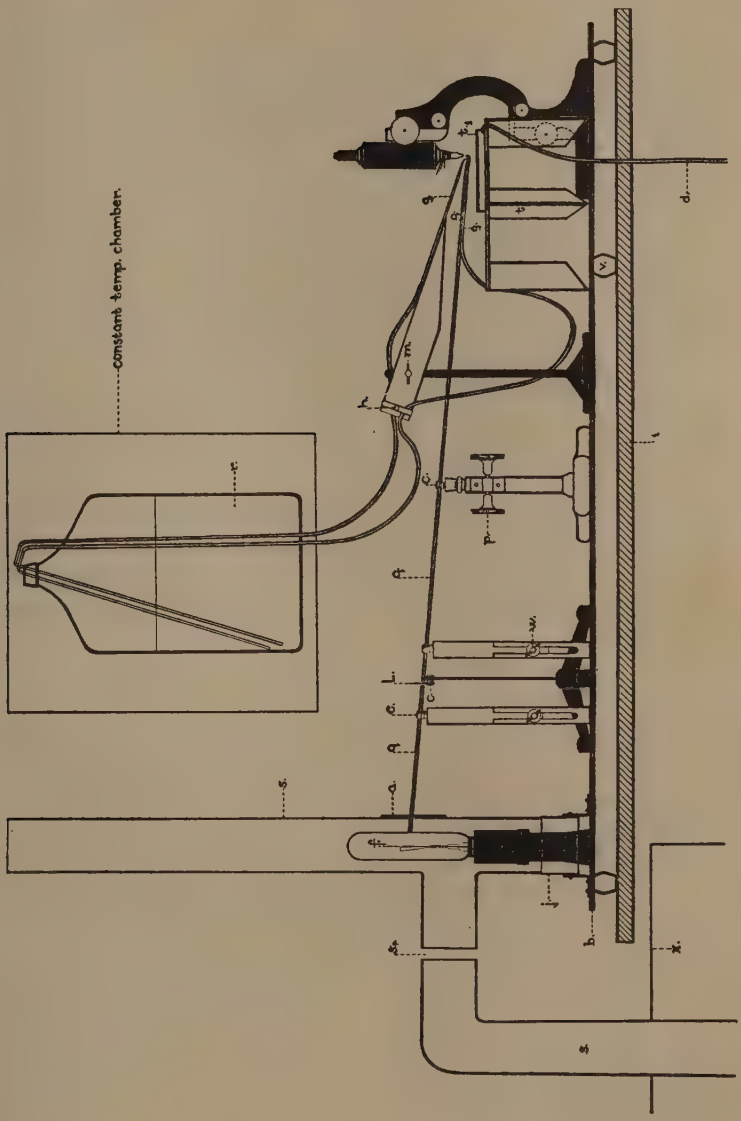
5. METHODS OF INCREASING THE LIGHT-CONDUCTING EFFICIENCY OF FUSED QUARTZ RODS

At several places in a fused quartz rod a fraction of the light which is passing that place is lost. Some of these places are at curves and bends in the rod, and at wrinkles in its surface, at the break in the rod where light filters are inserted, at the ends of air bubbles in the fused quartz, at the concentrating segment, and at soiled spots, especially finger prints, on the rod's surface. The total losses from a combination of such places can greatly reduce the light-conducting efficiency of a rod.

Smooth, polished, relatively wrinkle-free, optically-clear, machine-made, fused quartz rods can be obtained.[7] They

[7] The rods may be obtained from Thermal Syndicate, Ltd., Schenectady and Atlantic Avenues, Brooklyn, N. Y., and from Amersil Company, Inc., Chestnut Avenue, Hillside, N. J.

Fig. 5 One form of tissue illuminating apparatus. a, asbestos sheet which fits snugly around the quartz rod to prevent light from escaping into the darkened room; b, boiler plate base $\frac{5}{16}$ in. × 24 in. × 30 in. (0.78 × 60 × 75 cm.). Heavy base is used to diminish vibration of apparatus; c, clamps made of spring clothes-pins to permit easy removal of rod to clean it; d, drain tube to carry waste fluid from the drip tray; f, filament of 400-watt 'T 10' bulb; g and g, glass tubes which bring Ringer's solution to tissue; h, screw clamp which regulates flow of Ringer's in one tube. There is a similar screw clamp on the opposite side to control fluid flow in the other tube; i, top of work table; j, joint in stove pipe so that the upper part can be easily removed to clean bulb, etc; l, light filter inserted in the break in the rod. It is held in a clamp fastened in a movable stand; m, movable stand by means of which one controls exact position of upper glass tube tip g; p, pinion and rack of old dissecting microscope stand, used to secure careful control of elevation and lateral movement of quartz rod tip. It usually stands much closer to the microscope than is here pictured; q, fused quartz rod about $\frac{1}{4}$ inch (6 mm.) in diameter; r, Ringer's solution bottle; s, stove pipe; sp, space, so that vibration from the electric fan is not transmitted to the apparatus on the boiler plate; t, three-legged table which acts as microscope stage. After the specimen is prepared for study it is not touched or disturbed. The microscope is moved around to get different views of various parts of specimen. The microscope has its stage removed; v, two rubber stoppers with wide faces cemented together, used as leg under the boiler plate to isolate it from building's vibrations; w, wing nuts. By loosening them one can adjust the height of the clamps that hold the fused quartz rod. A similar arrangement permits lateral movement of the clamps; x, box which acts as a compression chamber. An electric fan blows air into the box. The air the fan sends into the box is vibrating due to the forward 'pats' the fan blades give the air at a given position as they pass by. The box is large enough ($\frac{1}{8}$ cu.m.) so that air coming out comes in a steady stream; thus the vibration of the air from the fan does not shake the light bulb.


constant temp. chamber.

contain some air bubbles, but are at present more efficient for light conduction than are hand-made rods. (The hand-made rods contain fewer bubbles than the machine-made, but whatever efficiency is thus gained is more than lost because of the number of wrinkles in the surface of the hand-made rods.)

Because each rewarming and reshaping of a rod adds wrinkles to its surface, it is well to bend it to the desired shape at a single heating. (An oxygen-fed flame is necessary to bend fused quartz rods.) No more bends are made in a particular rod than are required. The rods may be washed with soap or cleaning powder and water, rinsed in distilled water, and dried on lint-free cloth to remove dust and finger prints.

At times a few drops, or a stream, of the Ringer's solution on the sides or the tip of the rod lets out considerable light. This is particularly annoying if the solution is dropping, because then the microscope image becomes darker and darker as a drop is being formed, and suddenly bright as the drop falls off the rod. A small wick of absorbent cotton hung on the rod conducts this stray wash solution to the drip tray eliminating this loss of light and the flickering of the microscope image which accompanies it.

6. MECHANICAL INTEGRATION OF THE PARTS OF THE APPARATUS[8]

In designing apparatus the main objective has been to make each adjustable feature such that the tissue to be studied and the whole animal are disturbed as little as possible during the various adjustments.

The rod is divided into two segments by the break (at L in fig. 5). Each end of the segment nearer the lamp is held in a clamp which can be adjusted vertically and laterally. The other segment has its base held in an easily adjustable clamp. The vertical movement of the rod's delivery tip is controlled by the fine rack and pinion of a discarded dissecting microscope stand. The lateral movement of the delivery tip is

[8] It may seem that this problem hardly needs to be discussed, but it is nearly always encountered when designing a special adaptation of the method for some particular purpose.

controlled by moving this stand back and forth manually. (Two and three-dimension rack and pinion sets have been used to control the position of the delivery tip. They are convenient but not essential.) Ordinary spring clothes pins with wooden jaws have proved to be most satisfactory for gripping the rod, because they do not grip tightly enough to break the rod, as metal, screw-controlled clamps sometimes do.

Mammals rest upon a screen or upon wooden ridges in the drip tray which support the animal above the level of the waste solutions. Amphibians rest on the bottom of the drip tray. The latter is lined with paraffin or rubber cement, to make it water-proof, and to insulate its surfaces so that its metallic parts do not act as electrodes in the Ringer's solution. The screen in mammalian drip trays is similarly insulated. A wedge under one end of the drip tray tilts it and so controls the rate of escape of waste solutions.

The drip tray stands upon a small three-legged table which serves as a microscope stage. By moving the drip tray and table with one hand and controlling the elevation of the delivery tip of the rod with the other, the delivery tip is brought into suitable relationship with the tissue to be studied. With practice this can in many cases be done without touching the tissues with hands or instruments.

By having the animal supported on the three-legged table, the microscope is left free to be moved about in tracing out the relationships of structures. (If one attempts to have the animal rest on the microscope stage, then the animal and delivery tip must be moved simultaneously, which requires too much attention to be convenient.)

As has been said, one of the glass tubes is fastened to the delivery tip of the rod in such a position that it directs a gentle flow of fluid between the tissue and the delivery tip. This tube of course accompanies the delivery tip wherever it is. The other glass tube is supported by an independently movable stand so that its flow can be directed wherever desired.

The experiment is least disturbed if each article (delivery tip, drip tray, three-legged table, microscope and glass tube

stand) is independently movable. After a chosen area is suitably illuminated, moving the microscope from field to field is the only mechanical adjustment necessary. The animal on its table is left entirely alone.

7. CONTROL OF VIBRATION

This consists of control of the parts of the apparatus, and of the more or less cyclic movements of the tissue under observation.

Apparatus. In controlling the vibration of the parts of the apparatus the usual procedures have been employed, such as a) selection of a room in which to work that is approximately free from vibration. b) Mounting the main part of the apparatus on a heavy base supported on rubber. The inertia of the base is great enough so that it is not set in motion by transient vibrations in the room. The parts of the apparatus are heavy enough so that small tremors do not disturb them. c) Isolating parts of the apparatus which produce vibration from the part which one wishes to be vibration free. The stove pipe which conducts air to cool the bulb has a break in it (at Sp in fig. 5) to prevent the vibration of the fan from being conducted to the apparatus. d) Breaking up vibrations which tend to reinforce each other. The column of air coming from an ordinary electric fan vibrates longitudinally. This longitudinal vibration is transmitted to the light bulb, thus causing a lateral vibration of the rays of light in the fused quartz rod. This makes the microscope image vibrate even when the rod, the specimen and microscope are stationary in relationship to each other. To eliminate this, the fan blows air into a box which is large enough so that the air column coming out of it does not vibrate enough to disturb the lamp bulb.

Movements of the tissue under observation

An animal's cardiac and respiratory cycles produce characteristic cyclical movements of many of its parts. Study of the movement of an organ usually shows that it consists of a combination of a back and forth movement with a rotational

vibration. Sometimes both of these can be considerably reduced by placing the animal in a suitable position. The organ to be studied can be partly supported and steadied by gently moving adjacent viscera against it. The effect of the rotational vibration can be reduced or eliminated by selecting for study those areas near or at the axis of rotation.

But in many cases the movement cannot be entirely eliminated. As Wearn, et al., ('34) state, one soon learns to observe continuously a constantly moving field. After some practice it is done unconsciously and the problem of the movement of the tissue becomes less important.

I prefer not to pin an organ down to study it for the reasons mentioned in the next section.

8. PRESERVATION OF NORMAL PRESSURE RELATIONSHIPS

In the study of the peripheral vascular system, the normal functioning of which depends upon the maintenance of normal pressure gradients, too much care cannot be taken in attempting to preserve the normal pressure relationships.

Consider two points on a capillary sufficiently close together so that both can be included in a single microscope field. The difference in blood pressure between the two points, which, if expressed in conventional pressure units would appear to be insignificant, is in reality the force which moves the blood in the capillary from one of those points to the other. Apparently insignificant experimental manipulations may create alterations in pressure within an organ which are greater than the small pressure differences which cause the normal movement of the blood in its small vessels. Even though the increased resistance to blood flow in a partially compressed capillary, or capillary bed, raises the pressure on the arterial side of the compressed area enough so that blood is forced through the capillaries of the partially compressed area, and even though this kind of phenomenon frequently occurs during normal pressure changes in some normal tissues, it has seemed preferable in attempting to study normal circulatory phenomena to study tissue into which such processes have not been introduced experimentally.

So in this work especial effort has been made to prevent experimental mechanical interference with capillary pressure gradients. The fused quartz rod and the glass tips of the wash solution tubes are not permitted to press against the surface or the mesenteries of an organ which is being studied. In the best experiments the fused quartz rod and glass tube tips are brought so close to the organ that the surface tension of the wash solution carries it to the tissue but the rod and glass tips do not themselves touch the living organ.

During physiological processes some organs are stretched more than others. Unusual stretching of the tissue of a given organ is avoided because an increase in one or two dimensions of the elastic tissue causes a decrease in the magnitude of, and concomitant pressure increase along the third dimension, and stretching the parts of some organs (or their mesenteries) initiates reflexes which affect the organs' vascular systems.

A coverslip over the surface of the organ being studied is not used, for the surface tension and atmospheric pressure (or manual application of pressure) which flattens a normally curved surface of a somewhat rigid organ is sometimes great enough to alter the normal pressure relationships of the capillary system of the organ.

9. TESTING THE METHOD

Early in the use of this apparatus several facts became apparent. First, that because the living tissue was being studied through the microscope, and the precision of observation thus greatly increased, it was necessary to be exceedingly careful to prevent the methods of handling the living tissue from disorganizing the structures and processes one wished to observe. Anesthetics and surgical procedures from which an animal can easily recover, and experimental procedures which give no gross indication of change in the animal or organ, very frequently cause alterations in the processes which can be seen by the aid of the microscope.

Secondly, it became apparent that some processes which are normal in one organ under one set of conditions can be decidedly abnormal in other organs under the same or other sets of conditions. For example, fluid rapidly passes through frog kidney glomerular capillary walls as a normal process (one can see that blood cells are closer together in the efferent than in the afferent), but the rapid passage of fluid through the walls of the capillaries which nourish smooth muscle constitutes one of the steps in the process of pathological stasis in the vessels, usually initiated by careless handling of the tissue. How could one tell whether an observed process was normal or pathological in a given organ under a given set of conditions?

Thus it became apparent that before any set of observations on the circulatory system of a given organ could be accepted as representing normal, or nearly normal, conditions, it would be necessary to make careful studies of several organs under a variety of conditions.

Test observations were begun by studying the web of the foot of untraumatized unanesthetized frogs, and tails of amblystoma larvae, comparing the processes observed with those described by Prof. August Krogh in his monograph on The Anatomy and Physiology of Capillaries. Portions of superficial skin circulation in ears of young mice were also studied without the use of anesthetic or trauma. Different anesthetics were tried, including pithing of the amphibia. Different amounts of cutting and clamping of tissue were tested to see how much that affected the circulation in other parts of the animal. Effect of blood loss was observed. These test observations and comparisons were extended to include the study of the circulation in such amphibian (frog and amblystoma) organs as brains, peripheral nerves, mesentery, stomach, gut, mesonephros, ureter, bladder, ovary, oviduct, lung, striated muscle, liver, tongue, suprarenal, paraphysis, and the valves and vasa vasorum of the conus arteriosus of the frog's heart.

In white and gray mice studies testing different kinds of variations in the method have been made upon the circulatory systems of the stomach, gut, mesentery, ovary, corpora lutea, uterus,[9] placenta, striated muscle, peripheral nerves, liver, lungs, pancreas, lymph nodes and spleen. Several of these organs have also been studied in rats, cats and guinea pigs.

The purpose of these test experiments has been to determine the conditions under which one could begin to believe that the observations made represented normal, or nearly normal, processes. The 4-year period of these preliminary test studies has served to build up a set of observations against which to test each new set of observations.

Zweifach ('34), in his description of his experiments, says: "If a mesentery preparation, in which the capillaries were constricted, was allowed to remain 10 to 15 minutes with the microscope illumination striking it, capillary dilatation always took place." He does does not state how his preparation was illuminated.

To test the effect of light upon capillaries and other small vessels, a set of experiments was performed consisting of four parts:

1. Various organs, such as mesentery, gut, stomach, uterus, liver, frog kidney, peripheral nerves (and others) of amphibians and mammals have been studied under different degrees of brightness of illumination with white light, namely, total visible spectrum, beginning with such dim light that structures could not be discerned, and increasing the illumination by slow degrees with a time lapse after each increase, until the tissue was so brilliantly illuminated that it was uncomfortable to look into the microscope. If the proper precautions were taken to maintain the normal tissue temperature, that is, if the temperature and rate of flow of the wash solutions were correctly adjusted, no changes in the routine processes of capillary circulation of illuminated areas could

[9] Compare description of the control of circulation at placental sites preceding, during, and following parturition in the gray house mouse uterus, M. H. Knisely, '34.

be observed, no matter how dimly, how brightly, or for how long the tissue was illuminated. The usual types of intermittent circulatory processes continued. The capillaries of a given illuminated area did not all dilate, as would be expected from Zweifach's description. Further, after brilliantly illuminating single small areas of thick tissues, as spleen, for 10 to 12 hours, or longer, and then turning the tissue over to see if the vessels which had been nearest the delivery tip were dilated, it has been found consistently that these vessels were routinely proceeding through their intermittent circulatory cycles, not unusually dilated, just the same as the vessels in other parts of the same organ which had been but dimly or briefly illuminated.

2. A set of gelatin light filters (Eastman Kodak Company's A, B, C4, D, E, F, G, H and K) were employed to test the effect of different bands of the visible spectrum on capillaries. Experiments were carried out, using a band of the spectrum and varying the degree of brightness of illumination from very dim to very bright. No band of the visible spectrum has been found that produces any visible effect on capillaries.

3. It was feared that there was a capillary dilatation but that it was too small or took place too slowly to be observed without a standard for comparison, so the above experiments were repeated, using an ocular micrometer caliper to determine whether small dilatation, caused by illumination, takes place. No such dilatation has been found.

4. Tests have been made to determine what kinds of incidental stimuli do produce capillary dilatation. It can be initiated by such things as raising the temperature of tissue, or handling it, or producing too extensive incisions and trauma in other (even distant) parts of an animal.

It must be pointed out that in this group of experiments the criterion of the test has been whether or not capillaries dilated. Tests made using this criterion do not of course tell what the effect of light is upon other factors of physiological processes.

10. EXAMPLE OF USE OF THIS METHOD

Part of Roofe's ('35) study was made upon living brains transilluminated by this method.

To study the dorsal surface of the Amblystoma brain, the overlying skin, muscle, and bones were carefully removed without loss of blood. In some experiments the semitransparent dura was not disturbed, in others it also was removed. The delivery tip of the fused quartz rod was placed in the animal's mouth with the tip pointing upward. The glass tube accompanying the fused quartz rod conducted plain water between the roof of the animal's mouth and the rod's delivery tip. The other glass tube conducted amphibian Ringer's solution slowly over the exposed surface of the dura, or dorsal surface of the brain if the dura had been removed. A gentle flood of plain water along the sides of the animal diluted and removed the waste Ringer's solution fast enough so that the animal's skin was not exposed to a solution hypertonic to it. (In the studies of the gross distribution of larger vessels, this latter was not necessary.) The Ringer's solution and the plain water were at room temperature.

By the use of this method the brain could be studied in its exact normal location. Surgical procedures were reduced to a minimum because the light from the fused quartz rod tip in the animal's mouth was bright enough to transilluminate the mucous membranes, bone, meninges, and translucent brain tissue between the roof of the mouth and the more dorsal blood vessels of the brain. Because the light brightly illuminated the translucent brain tissue, it was also possible, by use of long focus objective lenses, to study vessels lying somewhat below the surface of the brain.

Appropriate minimal dissections made it possible to study the vascular system of the lateral and ventral portions of the brain.

DISCUSSION

The three most important disadvantages of the method in its present form are: 1) That some surgical procedure is necessary if one is to study the internal organs of animals which have opaque skins; 2) that an anesthetic is necessary because of the surgery; and 3) that Ringer's solution has to be used over the surface of a tissue that is being studied. These three factors tend to disrupt normal physiological processes and their effects must be taken into account in studies made by this method.

At present the method permits acute experiments only. A single structure can seldom be observed for more than 10 or 15 hours. Compare this with the rabbit ear chamber method (Sandison, '28; Clark, et al., '30), by means of which tissue of an unanesthetized animal can be observed over a period of weeks or months. The brevity of the period of observation has so far prevented studies of growth changes except in one or two cases during the developmental periods when the changes in a given detail of structure were very rapid.

The fused quartz rod method of illumination has several distinct advantages: 1. Previously inaccessible structures can be illuminated for study. It seems probable that suitable care in designing surgical approaches will make it possible to study physiological, pharmacological and pathological processes of almost any tissue or organ of the smaller laboratory animals with a microscope while the tissue is alive, with its vessels, nerves and attachments intact.

2. The extent of surgical and traumatic procedures employed can be greatly reduced by suitable use of the rod. How closely the normal is approximated depends in part upon the plan and execution of the surgical approach employed. In designing the approach advantage is taken of the possibilities of inserting a rather long crooked rod in a body cavity or hollow viscus. Advantage is also taken of the fact that it is possible to send light through several layers of tissue to illuminate the structure to be studied. These two factors make it possible to have the site of part of the trauma at some distance from the structure to be studied.

3. Relatively opaque or thick translucent structures can be transilluminated. Observation of structures below the surface of an organ is limited, however, first by the focal length of the objective employed, and secondly by the capacity of the tissue (between the focal plane of the objective and the objective) to diffuse light. This factor varies in different tissues.

4. The angle at which the light strikes the tissue can be controlled. Reflected light, 'low incident light,' and transillumination are available.

5. As far as our present knowledge of tissue temperature permits us to judge, the control of temperature is adequate for many purposes.

Because the circulatory system is particularly easy to observe in living organs, owing to the refractility of leukocytes and of the lining of vessels and to the presence of natural pigment in erythrocytes—so the system can be studied without the use of dyes—much of the living tissue work done so far has been directed toward studying circulatory phenomena. For similar reasons this system has been used for testing the method. In its present stage of development, this method when carefully handled causes a minimum of interference to the circulatory system of the organ being studied. One can observe circulatory conditions and processes which routinely take place while the peripheral vascular system is acting under the control of its own internal regulatory mechanisms, and one can observe also the reaction of the system to external, experimentally produced stimulations.

Krogh ('29) shows that the total vascularity, the pattern of the finer vessels, the volume of blood supplied to, and the condition of blood flow in a given tissue, are intimately related to the structure and functioning of that tissue. This concept of the integration of the structure and functioning of the peripheral vascular system of a given organ with the other structures and functions of that organ, is a key to the study of the peripheral vascular system.

The essentials of the fused quartz rod method are rather simple. But, in common with other techniques, trustworthy results are obtained only at the expense of practice and patience.

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A STUDY OF THE THYROID IN EMBRYOS OF ELEUTHERODACTYLUS NUBICOLA

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TWO PLATES (ELEVEN FIGURES)

INTRODUCTION

The Amphibia typically exhibit two distinct periods in their life history; an early aquatic larval stage and a later terrestrial adult stage. The larva differs from the adult in many features, both morphological and physiological, and the transition from the one to the other constitutes a striking metamorphosis characterized by loss of the gills, shortening of the digestive tract, growth of limbs and lungs, protusion of the eyes, and in the anurans, loss of the tail. These radical changes do not occur spontaneously in the tissues at a particular time but are under hormonal control. The work of Gudernatsch ('12, '14) and of many others has shown that it is the secretion of the thyroid gland which is most important in bringing about metamorphosis; for larvae treated with extract of the gland metamorphose precociously while thyroidectomized larvae entirely fail to metamorphose unless the extract is administered to them. However, another endocrine organ also plays an important part in these processes, for removal of the pars anterior of the hypophysis prevents the occurrence of the metamorphic changes even when the thyroid is present (Allen, '16; Smith, '16). The interrelations between the activities of these two glands have been studied by many investigators. Removal of the thyroid is followed by hypertrophy of the hypophysis (Hoskins and Hoskins, '19; Smith, '20) while in the absence of the hypophysis the thyroid remains rudimentary (Adler, '14; Allen, '16, '17; Smith, 16, '20;

Hoskins and Hoskins, '19). Administration of extracts of the absent gland in either of these conditions tends to restore the remaining one to its normal state. Uhlenhuth and Schwartzbach ('27) have concluded that the pars anterior of the hypophysis exerts its influence upon metamorphosis indirectly by affecting the growth and differentiation of the thyroid and this view has gained general acceptance.

It must be emphasized however, that any change which occurs at metamorphosis depends not only upon the hormone which calls forth the change but also upon the nature of the tissue acted upon. Within the same organism some tissues change radically in response to the thyroid secretion while others remain unaffected; others are affected in varying degrees. Moreover the same type of tissue in different organisms reacts differently to the hormone influence; so that, what occurs in a tissue depends in large measure upon its hereditary constitution and not merely upon the presence or absence of a particular hormone. In addition to this, the various amphibia differ greatly as to the degree of differentiation which they are able to reach before the hormonal influence is necessary to induce further differentiations. Thus if the larvae of Rana pipiens are thyroidectomized the hind limbs will appear as buds but differentiate no further; in thyroidectomized larvae of Ambystoma microstomum the hind limbs differentiate further and the fore limbs also appear; in Bufo americanum larvae, similarly treated, the hind legs attain a length of 7 to 8 mm. Similar relations are found with respect to other tissues and it appears to be a general rule that for every tissue affected there is some critical stage at which the intervention of the thyroid influence is necessary for the completion of metamorphic changes. This critical stage is comparatively early in some species and when these are deprived of their thyroids they remain in a relatively undifferentiated larval state throughout life. In other species however, the critical stage is later and these may attain a relatively high degree of differentiation in the absence of the thyroid.

The facts outlined above relate to the usual type of amphibian life history such as that of the common frog. However, knowledge of the interaction of glandular secretions and tissues in normal metamorphosis has directed attention to those species of amphibians in which there is some marked deviation from the usual type of life history and much study has been given to those forms in which metamorphosis is long deferred, incomplete or, as in Necturus and Proteus, entirely lacking. The question naturally arises as to whether in these cases the cause of the failure to metamorphose is to be found in the glandular mechanism or in the tissues themselves. Is it the result of some abnormality in the structure or development of the thyroid or hypophysis in these animals or are the tissues which usually undergo alteration at metamorphosis constitutionally incapable of responding to the thyroid influence? In other words, is the altered life history the result of some change in the glandular mechanism such that the characteristic secretion is no longer produced or no longer released, or is it the result of some change in the tissues such that they no longer respond in the usual way to these substances? This question has been subjected to experimental investigation in a number of cases and it appears that in different forms each of the alternatives stated above is responsible for the failure to metamorphose. The Mexican axolotl usually retains the larval form throughout life and no metamorphic changes occur. However, the animal's tissues are capable of differentiating to produce a salamander of typical adult form and can be induced to do so by thyroid administration. Moreover the animal's own thyroid is functional and elaborates a secretion capable of inducing metamorphosis in frog tadpoles (Swingle, '22). It appears likely that in this case the failure to metamorphose is due to some defect in the release mechanism which prevents the hormone from reaching the tissues at all. On the other hand, in forms like Necturus, although the thyroid is functional and can be induced to discharge its colloid (Grant, '30) the tissues show no response to the hormone and seem incapable of undergoing the typical metamorphic

changes. They remain permanently in a relatively undifferentiated larval condition.

Now, the great interest which attaches to the forms just discussed would seem to apply equally to another group of amphibians in which the life history is just as atypical. This is the group of species in which the larval stage is passed through rapidly, in a most abbreviated form and the animal emerges from the egg with the definitive body form. Such amphibians thus omit the early aquatic stage of their life history and may be contrasted with the perennibranchiates in which the terrestrial adult stage is lacking. There are a number of genera and species among both the urodeles and the anura in which such a condition obtains and in many of them the deviation from the usual type of life history appears clearly to be of adaptive significance. In some, the eggs are retained within the body of the mother and the young are born with the adult form, in others the eggs are laid on land and there develop into the young frogs or salamanders. Among the different species which exhibit this habit, great variation exists as to the degree to which ordinary larval organs are developed. In some cases external gills appear, in others there is no sign of them, and there is a similar variation with respect to development of limbs, protrusion of eyes, loss of the tail, etc. However this may be, it is obvious that a great modification has occurred during the evolutionary history of these groups as a result of which the usual larval structures appear only transiently or are entirely lacking; and metamorphosis, if it may be spoken of at all, occurs very precociously. Now in view of our knowledge of glandular function in normal metamorphosis, it seems possible that this evolutionary change may have been brought about either by a change in the development and functioning of the gland complex, or by a change in the tissues themselves. Thus, the same interesting questions arise here as were considered with relation to those forms whose metamorphosis, presumably by a similar evolutionary modification, is delayed or inhibited. Is this early tissue differentiation and assumption of the adult

body form associated with precocious development and functioning of the thyroid and hypophysis or is it the result of an hereditary constitution which enables the tissues to undergo complete differentiation to the adult form without the influence of the thyroid? Are the evolutionary changes which have brought about the atypical life history of these species chiefly changes in the development and functioning of the gland complex or chiefly changes in the tissues usually affected at metamorphosis, enabling them to differentiate fully in the absence of the hormonal stimulus?

The present paper is an attempt to obtain some light upon these questions by a study of the thyroid and hypophysis in the embryonic stages of one of the anurans, Eleutherodactylus nubicola, which exhibits this type of direct development.

MATERIAL AND METHODS

The material used for this study was collected in Jamaica, British West Indies, during the summer of 1932. The eggs were found in small hollows under stones along the trail between Morce's Gap and St. Helen's Gap near the Cinchona plantation. This trail follows almost exactly the 5000-foot contour line. In many cases the adult was found with the eggs and served for later identification of the species. The number of eggs in a clutch averaged 44 and varied from 40 to 53. It appears that there is no sharply marked time of egg deposition since in eight groups of eggs collected within a few hours on June 26th all stages of development from late cleavage to hatching are represented and, in fact, eggs in various stages of development were collected throughout the summer. Since all the embryos in any one clutch proved to be very nearly in the same stage of development, many series of sections of any particular stage are available. All the embryos were fixed in Bouin's and preserved in alcohol. They were later sectioned and stained with various stains; Mallory's triple connective tissue stain and haematoxylin and eosin being most used. Although the widest range of embryonic stages is available for study there are unfortunately

several desirable ones which are lacking. However, four stages ranging from the first appearance of the limb buds to the time of hatching have been selected for description here. The embryology of Eleutherodacytlus has been well worked out by L. V. Sampson ('04) and the stages used in the present study may be conveniently identified by reference to the embryonic stages discussed and figured in her paper. Thus the first stage to be described corresponds to Miss Sampson's stage II; the second corresponds to her stage V; the third to her stage X; and the last to stage XIV, the time of hatching.

OBSERVATIONS

The first stage studied is that shown in figure 1. At this time both fore and hind limbs are present as buds and the tail is just beginning to be distinguishable. Internal differentiations have not proceeded far. The neural tube is closed and the three primary divisions of the brain are established. The optic vesicle is apparent as a diverticulum from the brain and the otic placode can be distinguished opposite the hind-brain. Although the embryo at this time shows some signs of the beginnings of visceral pouches nothing which could be interpreted as the anlage of the thyroid could be found.

The second stage (fig. 2) shows a considerable advance in development in a number of respects. The increase in size of the whole embryo is accompanied by growth of the limb buds and of the tail. The brain is further differentiated, the definitive divisions of the brain now being marked off, although the hypophysis is not yet established. The optic cup has been formed and encloses the lens which has just been cut off from the surface. The otic placode has sunk in to form the otic vesicle and the diverticulum which is to become the endolymphatic duct has developed. The heart is still a tubular structure at this stage. The visceral pouches are still present and the tracheal groove has been formed. It was hoped that this stage might afford some evidence on the establishment of the thyroid but careful study of a number of series of sections showed no trace of such a structure. The two stages just dis-

cussed may thus be dismissed briefly. They have been described because they demonstrate the establishment and differentiation of several important structures which originate and develop before the glands which usually have to do with metamorphic changes have been formed at all.

The third stage to be discussed is illustrated in figure 3. It is clear that this represents a considerably older embryo and that other stages between this and the preceding would be most desirable. The embryo has now assumed the adult body form and the body has become slightly pigmented. The hind limbs have increased greatly in size and the joints of the toes are differentiated. On the fore limb the digits are represented by buds. The dermal fold which covers the base of the fore limb and which may possibly be the homologue of the operculum is clearly visible. The tail has become a large unpigmented, richly vascularized sac which is assumed to function as a respiratory organ; it may be seen in the photograph covering the hind limbs almost completely. The brain and organs of special sense now show differentiation of all definitive parts. The hypophysis is established and the anterior lobe shows the presence of both oxyphil and basophil cells when treated with Mallory's triple stain.

In embryos at this stage the thyroid gland is present as a paired structure composed of small vesicles containing colloid (fig. 6). Figures 8 and 9 show sections through typical vesicles of such an embryo. The epithelial cells are in a single layer and are flattened to cuboidal in form. The colloid mass within the lumen of the follicles stains a uniform blue with Mallory's triple stain and shows no vacuolation although shrinkage of the colloid is sometimes apparent. No colloid droplets were ever observed in the vesicle cells themselves. Judging by the form of the lining cells and the staining reaction of the colloid this seems to present a picture characteristic of primary thyroid follicles containing freshly produced colloid and in a relatively inactive state.

The final stage (fig. 4) is the time of hatching. The embryo is now a complete little frog. The tail has decreased considerably in size and will be completely resorbed shortly after

hatching. The operculum-like dermal folds have disappeared. Both fore and hind feet have all joints differentiated and the characteristic adhesive discs can be seen in the photograph. The egg tooth at the tip of the nose, which assists the animal in hatching may also be seen. The anterior lobe of the hypophysis (fig. 5) still shows both oxyphil and the basophil cells but upon comparison with the preceding stage no differences in the relative numbers of these can be found.

The thyroid glands have increased in size and present a much changed aspect (fig. 7). Many of the epithelial cells are now columnar in form with the nuclei at the bases of the cells (fig. 10). The staining reaction of the colloid is altered so that it is now colored red with Mallory's triple stain although a blue border of greater or less extent is usually present. The red center and blue border are well seen in figure 7. At this stage also vacuolation is absent. In a number of follicles the colloid mass may show several red centers completely surrounded by blue-staining substance. This may represent a stage shortly after fusion of several primary follicles. An example of such a follicle is shown in figure 11. It must be noted that there is considerable variation at this stage as to the histological picture presented by the thyroid. Although many of the follicle cells are columnar there are also a great many which retain the cuboidal form. Also the relative amount of red-staining and blue-staining colloid varies enormously in different individuals. The staining reaction of the secretion here indicates that it is stored colloid, for the picture obtained is typical of that described by Uhlenhuth ('27). Nevertheless it appears that we have some indication of increased activity in the gland at this time as evidenced by the appearance of columnar epithelium.

DISCUSSION

From the evidence at hand it appears that many of the differentiations which occur in correlation with thyroid activity in most frogs are accomplished in Eleutherodactylus independently of the functioning of that gland. The origin and differentiation of the limbs are processes which clearly go on before there can have been any release of the thyroid secretion. On the other hand active secretion and storage of colloid in the thyroid does occur at a very early age in these embryos and the signs of heightened activity of the gland which are observed in the last stage studied may indicate the onset of the release of the secretion. If this does take place at about this time it may be found to have some correlation with the disappearance of the dermal fold covering the base of the fore limbs and with the resorption of the tail; processes which taken together might be regarded as constituting as close an analogy to metamorphosis as can be found in this species. In any case the modification in the mode of life history which has arisen in these animals certainly involves changes in the hereditary constitution which make possible the attainment of an unusual degree of differentiation without the intervention of control by hypophysis and pituitary. Moreover the development has been so modified that many of the adult features which first appear at metamorphosis in most frogs are developed in this species without any trace of the usual intermediate conditions. It is obvious that the present study may only be regarded as preliminary and it is hoped that an opportunity may soon be afforded to make further collections in order to complete the investigation. Intermediate stages are much needed. Embryos between stages 2 and 3 are especially desirable since they would give the origin of the thyroid and the establishment of the primary follicles. Stages after the time of hatching must also be studied in an attempt to ascertain the time of release of the colloid. In addition experimental removal of the thyroid in these animals may be possible.

SUMMARY

1. In Eleutherodactylus nubicola the fore and hind limbs form and the digits are differentiated before the origin of either hypophysis or thyroid.

2. In the first stage of thyroid development studied, the primary follicles are established and secretion of colloid has begun.

3. The changes in staining reaction of the colloid follow their usual course, the colloid at first staining blue with Mallory's triple stain but later changing to red. The latter condition has been attained by the time of hatching.

4. From the material available it appears that, though active secretion and storage of colloid occurs during embryonic life there is no clear correlation of thyroid activity with any of the bodily changes in the embryo which may be interpreted as metamorphic in nature.

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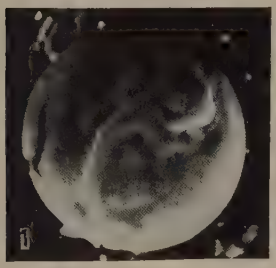
PLATE 1

EXPLANATION OF FIGURES

1 to 4 Four stages in the embryonic development of Eleutherodactylus nubicola. The outer coverings of the egg have been removed in all. × 10.

5 Longitudinal section of the hypophysis of an embryo at the stage shown in figure 4. × 400.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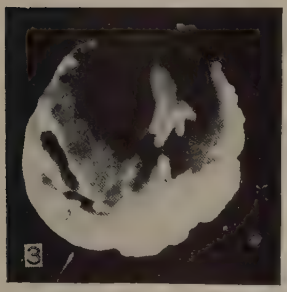
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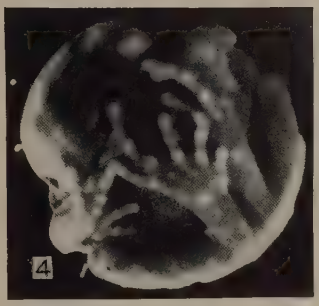
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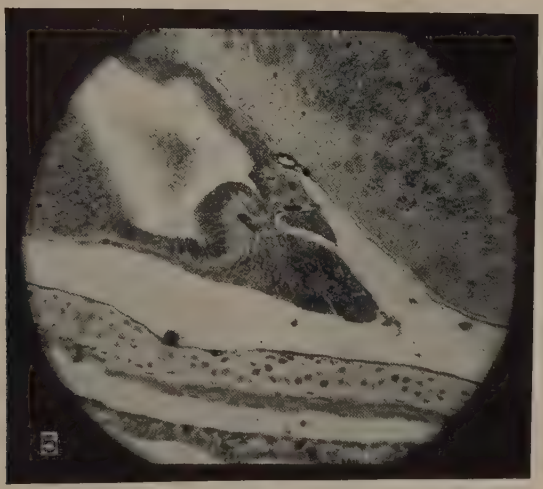
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PLATE 2

EXPLANATION OF FIGURES

6 Transverse section of thyroid gland of an embryo at the stage shown in figure 3. The colloid is stained blue and appears light in the photograph. × 300.

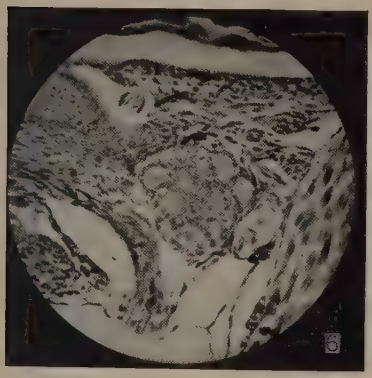
7 Transverse section of thyroid gland of an embryo at the stage shown in figure 4. The central portion of each colloid mass takes a deep red stain but shows a narrow blue outer border. × 300.

8 and 9 Single vesicles from thyroids of embryos at the stage shown in figure 3. The cuboidal lining cells and the blue staining colloid is clearly seen. × 1200.

10 Single vesicles from thyroid of an embryo at the stage shown in figure 4. The lining cells tend to be columnar in form and the colloid mass takes a red stain. × 1200.

11 Vesicle from thyroid of an embryo at the stage shown in figure 4. The colloid mass here shows three distinct red-staining centers each surrounded by a blue border. × 1200.

All preparations were fixed in Bouin's, sectioned and stained on the slide with Mallory's triple connective tissue stain.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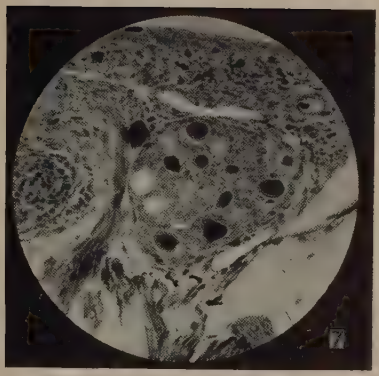
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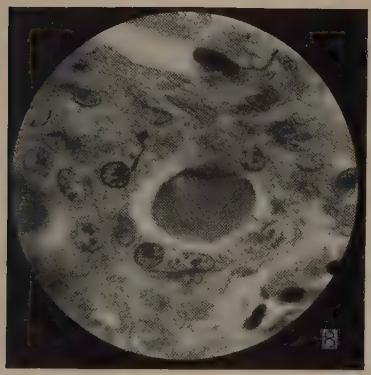
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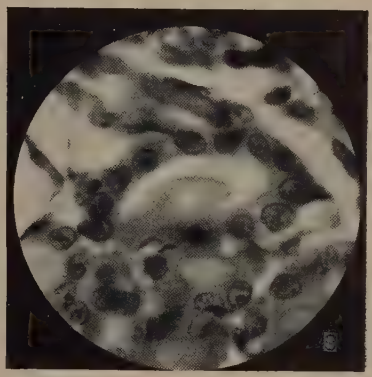
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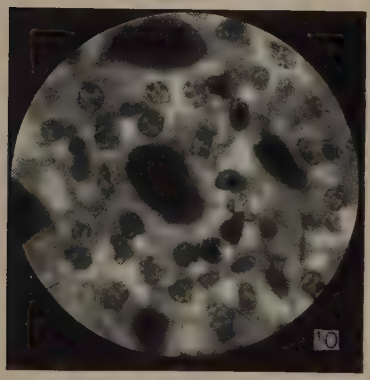
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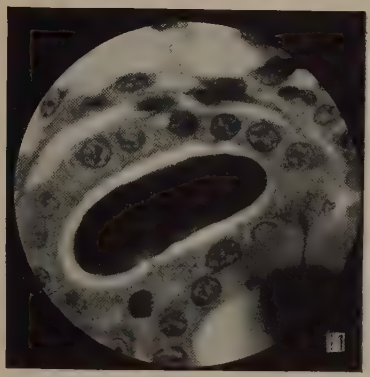
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NEW BOOKS AND PUBLICATIONS

THE CRANIAL MUSCLES OF VERTEBRATES, by F. H. Edgeworth. 1935. Size, 9½ × 13 inches. Pages 9 + 493. 841 illustrations. Index. Table of abbreviations. Table of synonyms. Bibliography. Cloth. Price, $30.00. The University Press, Cambridge, and The Macmillan Company, New York.

Genetic relationships of vertebrates and phylogenetic history of man in the remote past are studied in this survey of cranial musculature. Present day knowledge of comparative anatomy and embryology has been summarized to show primitive form and arrangement of muscles and lower motor neurons in each phylum, and to trace evolutional changes. An attempt has been made to give, wherever possible, identical names only to homologous muscles inherited from common ancestors, and to apply different names to homoplastic muscles resulting from convergence. The excellent illustrations take up 193 pages, and are arranged in order by phyla, from cyclostomata to mammalia. Other desirable features are the table of synonyms and the classified lists of bibliographic reference. The work is attractively published with clear type and wide margins.

CLINICAL MISCELLANY. The Mary Imogene Bassett Hospital, Cooperstown, New York. Volume 1, 1934. Contributors: Floyd J. Atwell, C. Lee Buxton, Francis F. Harrison, Monroe A. McIver, Charles C. McCoy, G. M. Mackenzie, Marjorie F. Murray, Robert M. Pike, John H. Powers, T. J. Abernethy. Size, 6¼ × 9¼ inches. 214 pages. 37 illustrations, some of them in color. Index. Bibliography. Cloth. Price, $3.00. Charles C. Thomas, Springfield and Baltimore.

This book contains a collection of selected clinical observations on rare diseases or cases showing unusual complications not often encountered by the practising physician. There are twenty-two chapters by various authors giving complete case histories and comments. They describe such widely separated entities as undulant fever, botulism, agranulocytosis, spontaneous subarachnoid hemorrhage, intestinal carcinoma, gunshot wound, and postoperative treatment of prostatic obstruction.

HANDBUCH DER NEUROLOGIE, edited by O. Bumke and O. Foerster. (Allgemeine Neurologie I. Anatomie. Band I.) 1935. Size, 6¾ × 10 inches. Pages 1152. 585 pages. Table of contents. Chapter bibliographies. Appendices. Price, RM 220; bound, RM 225. Julius Springer, Berlin.

This volume comprises thirteen chapters by outstanding workers in the field of neurology on the special subject of each. The general subjects are as follows: History of the Egg, by M. Rose, Wilno; General Histology and Histopathology of the Nervous System, by Alice Rosenstein, New York; Anatomy of the Spinal Cord, the Medulla Oblongata and the Bridge, by E. Pollak, Wien; Anatomy of

the Cerebellum, by O. Gagel, Breslau; Anatomy of the Mid-brain, by H. Spatz, München; Anatomy of the Cerebrum, by M. Rose, Wilno; Cyto-architecture and Myelo-architecture of the Cerebrum; by M. Rose, Wilno; Structure of the Blood Vessels, by L. Freedom, Baltimore; The Construction of the Joints of the Central Nervous System, by A. H. Schroeder, Montevideo (Uruguay); Microscopic Anatomy and Histology of the Developing Nervous System, by R. Greving, Schweinfurt; The Neurone Theory, by S. Ramón y Cajal, Madrid; Nervous Regeneration, by J. Boeke, Utrecht.

For the teacher and advanced student of neurology and neuro-anatomy, this collection is one with which to become familiar. For the research worker, one to use as reference.